ACTA UNIVERSITATIS SZEGEDIENSIS

# ACTA BIOLOGICA

NOVA SERIES

TOMUS XXI FASCICULI 1—4

SZEGED (HUNGARIA)
1975

ACTA UNIVERSITATIS SZEGEDIENSIS

# ACTA BIOLOGICA

NOVA SERIES

TOMUS XXI FASCICULI 1—4

SZEGED (HUNGARIA)
1975

Adiuvantibus
L. BOROS, L. FERENCZY, I. HORVÁTH, ERZSÉBET KÖVES,
L. MÓCZÁR, L. OROSZ, L. SZALAY

redigit
PÁL LIPTÁK

editionem curat
GY. BODROGKÖZY

Edit
Facultas Scientiarum Naturalium Universitatis Szegediensis de Attila József nominatae

Nota
Acta Biol. Szeged.

---

Szerkeszti
LIPTÁK PÁL

A szerkesztőbizottság tagjai
BOROS L., FERENCZY L., HORVÁTH I., KÖVES ERZSÉBET,
MÓCZÁR L., OROSZ L., SZALAY L.

Szerkesztőbizottsági titkár
BODROGKÖZY GY.

Kiadja
A Szegedi József Attila Tudományegyetem Természettudományi Kara
(Szeged, Aradi vértanúk tere 1).

Kiadványunk rövidítése
Acta Biol. Szeged.

Acta Biologica Szeged. *21* (1—4), pp. 3—8 (1975)

# PROF. PÁL GREGUSS IS 85 YEARS OLD

Dr. PÁL GREGUSS, Kossuth-Prize Winner, Professor Emeritus of Attila József University, Doctor of Biological Sciences, member of several Hungarian and foreign scientific societies, was eighty-five years old on December 31st 1974. At the celebration arranged in his honour he was warmly greeted by official functionaries, and by his pupils, acquaintances and friends.

In the first part of the celebration, the Rector of the University, Prof. GÉZA FODOR, addressed Professor GREGUSS in the senate-house of the University, presenting him with a letter of congratulation from the Minister of Education, Dr. KÁROLY POLINSZKY:

"Dear Professor,

Please permit me to offer you my sincere congratulations on the occasion of your eighty-fifth birthday. In paying tribute to your course of life and activity, I am greeting not only the Kossuth-Prize winning scientist, but the University professor as well, who has always been widely esteemed, and who enjoys a high reputation in the field of higher education for his exemplary educational work.

I wish you good health and a long life to be spent in delight and happy contentment. Yours sincerely: Dr. KÁROLY POLINSZKY".

Subsequently, in the flower-decorated biological lecture-room of the University a joint session was held in honour of Professor GREGUSS by the Szeged Division of the Hungarian Biological Society, the Biological Committee of Attila József University, and the Biological Committee of the Szeged Division of the Hungarian Academy of Sciences. The following address was first delivered by Dr. ANDRÁS GARAY, Corresponding Member of the Hungarian Academy of Sciences, President of the Szeged Division of the Hungarian Biological Society:

"Ladies and Getlemen, dear Professor GREGUSS,

Permit me to greet you in the name of each of the members of the Hungarian Biological Society, whether in Szeged or not, on the occasion of your eighty-fifth birthday.

After being informed that, as President of the Division, I had to deliver some words of greeting, I asked for the advice of several members of our Society as to what I should say in the frame of our good wishes. I felt this enquiry to be my duty as I had to express the congratulations of us all. The first reflection of everybody of course was, to wish you a long life to come rich in work. Following that, our colleagues mentioned the most varied things. Some of them emphasized the professional continuity and stability in your work; others accentuated, quite in contrast, your comprehensiveness and dynamism. I am convinced that both of these opinions are equally true. You have dealt with xylotomy for the sixty-five years of your industrious life, with undiminished energy and the greatest profoundness. This, however, did not prevent you from responding sensitively

to any novelty emerging even in remote fields. Only very rarely does one encounter a researcher capable of harmonizing continuity and devotion to a single subject with dynamism and a broad outlook. Overall, you have succeeded in achieving the harmony of the researcher and pedagogue in your life.

Another considerable aspect was frequently mentioned as well, namely that you have always regarded administration as of secondary importance and given your attention to the essence of the work. I think that this positive characteristic cannot be emphasized sufficiently in a period when so many problems are caused by excessive bureaucracy. I am convinced that beneath this seemingly minor facet a very important feature is concealed, with a gift for seeing the essence.

In expressing the best wishes of every member of the Hungarian Biological Society to you, therefore, I am expressing our gratitude as well, for promoting science considerably in a narrow field, and thereby winning renown for this country. Thanks to your educational activity, our thinking could never be narrowed down to a single line but was able to survey the whole horizon. I wish to express, therefore, the gratitude and congratulations of every member of the Hungarian Biological Society, wishing you a long creative life, and begging you to keep on teaching us in both educational and scientific respects".

Vice-Rector Dr. JÁNOS HEVESI next addressed the professor in the names of the Senate and the Rector of the University:

"Mr. Chairman, Ladies and Getlemen, dear Professor GREGUSS,

I was honoured with the request of Dr. KÁROLY POLINSZKY, Minister of Education, to read at this session his letter addressed to Professor PÁL GREGUSS. I should like to inform the audience that the Rector of our University has already handed the letter from the Minister to Professor GREGUSS and greeted him in the name of the University Senate as well.

I must end by again congratulating you, Professor GREGUSS, on behalf of the University Senate and in my own name too. I wish you that you may live and work for a long time to come in good health, doing honour to our University, and raising its scientific reputation. We are much indebted to you for your devoted pedagogic activity, and for educating a great number of well-trained teachers for this country. May your further work be accompanied in this area too by good luck and success".

Dr. GÁBOR FARKAS, Corresponding Member of the Hungarian Academy of Sciences, Vice-President of the Biological Division of the Hungarian Academy of Sciences, delivered the following address:

"Dear Professor GREGUSS,

Allow me to express my sincere best wishes on the occasion of your eighty-fifth birthday, in the names of the Division of Biological Sciences of the Hungarian Academy of Sciences and of the President of the Division.

The Hungarian Academy of Sciences and, within this, the Division of Biological Sciences have always followed your activity with great attention, interest and high regard, and have professed great esteem for it. Your love for, and devotion to scientific work may, even today, be a model for the young generation of research workers. We wish you to remain in good health a creative member of our scientific life, with similar enthusiasm and for many long years".

Dr. ERZSÉBET S. KÖVES, Vice-Dean of the Faculty of Science of Attila József University, a one-time pupil of Professor GREGUSS, greeted the renowned professor, scientist, teacher, and educator in the name of the Faculty of Science.

Dr. IMRE HORVÁTH, President of the Biological Committee of Attila József University, pupil of Professor Greguss, addressed the celebrated professor as follows:

"Dear Professor GREGUSS,

On the occasion of your eighty-fifth birthday I greet you in the names of the Biological Committee of Attila József University, the Department of Botany and the Botanical Garden and myself, as a one-time pupil.

In the course of a festive address it would be difficult to give an appreciation of your pedagogic and research work of more than sixty years. Permit me, therefore, to mention rather some questions of personal character. Professor GREGUSS has several qualities that are fundamental for achieving scientific results. I shall emphasize only three of these:

The first one: The ability to shape a scientific conception from a comprehensive amount of knowledge. Professor Greguss was capable of doing that nearly sixty years ago, putting forward the conception of the trifiletic evolutionary theory of the vegetable kingdom. (The phylogeny of sexuality and triphyletic evolution of the landplants.)

The second important quality is hard, consistent work, with no recoil from difficulties. Everybody who knows Professor GREGUSS is fully aware that these are his strong characteristics.

As a third quality I may mention the effective performing of scientific research, including participation in the detailed work, and not only in directing and the evaluation of experimental data collected by other persons. Even at present Professor GREGUSS himself prepares the majority of this sections and photomicrographs.

I don't think it more chance that not only Professor Greguss, but several of his pupils too, have achieved internationally outstanding scientific results. Twenty-five of these are Academicians, Doctors or Candidates in Biological Scienses. At least the same number have obtained [the University doctorate, many of them being University and College teachers and some of them chairholders and heads of departments.

I myself am proud to be a pupil of Professor Greguss, having made an effort to acquire from him the qualities mentioned a little earlier.

I am very pleased that in spite of his advanced age, Professor Greguss is at present continuing his scientific research work with undiminished energy from day to day, helping those who ask for his advice. I trust that he may work and create among us for many long years to come."

At the end of his words, he handed Professor GREGUSS a memorial chalice in the names of the Department of Botany and the Botanical Garden.

In conclusion, the celebrated professor was greeted with flowers by the representatives of the young people. After the addresses the Chairman, Dr. ANDRÁS GARAY, read aloud the special telegrams and letters of congratulation sent on the occasion of the anniversary of Professor GREGUSS.

Touched by emotion, Professor GREGUSS expressed his thanks for the congratulations, and then summarized the history of his scientific career of approximately seven decades. At the beginning of his speech he said the following:

"Mr. Chairman, Ladies and Gentlemen,

I should like to address my first words of thanks to those honouring me by arranging this celebration of my anniversary so generously, without forgetting their one-time colleague, co-worker, and teacher even ten years after my retirement. They may all be convinced that I have not forgotten any of them, either; I remember each of them always and with the old pleasant feelings. For the present commemoration I should like to express my most grateful and sincere thanks.

I have always declared, and do this at present too, that only a dull and self-conceited man disclaims honour and love. In my opinion, apart from health and knowledge, love is the most precious treasure in the world, and love may only be a reward of loving.

Looking at this illustrious meeting, I feel as if I were at a trial by jury where there are barristers and attorneys; with their legal parlance, these have over elaborated the data of my life and activity so far and have tried to justify why they assigned a place to an eighty-five years old teacher at the table of honour and recognition. In their pleadings they magnified my possible virtues and results, but were silent concerning my many faults and errors. I wish to record my best thanks for their kind addresses.

Also here are the members of the jury, our guests, who on the basis of the pleadings of the barristers, will cast their votes as to whether this man of eighty-five has deserved this honouring session, how he has fullfilled his duties, and how useful he could make himself for his country, family, neighbours, and the large number of his pupils. I am grateful to them for their attendance.

Being here myself as the accused, as it were, I ought to avail myself of the privilege of the last word for defending myself. I have a feeling that I should give the reasons for my being here at this trial by jury; that I have to reveal my whole life, showing where I started from, and the very thorny and rugged paths along which I stumbled to attain various high positions, finally at the age of eighty-five, being asked to take a seat at the table of distinction. When, as an interested party, I try to do all this, I must apologize in advance for viewing the events connected with my person in a perhaps somewhat subjective way. However, I should like to demonstrate in this way, mainly for my pupils

and young people, that it is worth while to set ourselves high targets in our early youth and to remain faithful to these even under the worst conditons, till in the end we reach the objects of our lives. I feel fortunate that I have attained most of the ambitions set in the days of my early youth. For that success, I am indebted first of all to the practicability of my aims, to have love of the natural sciences, to my health, and to my always honest work. These have led me during my whole life, giving it beauty and meaning."

Following this, Professor GREGUSS surveyed the course of his life, emphasizing his major scientific results. This part of his address is reported below in a shortened form.

He was born in Tornya on December 31st 1889. His father was a village master-joiner with eight children, who worked later as a journeyman-joiner in the carriage and waggon works in Arad. At the beginning, he took part in the work in the joinery of his father, and then, after leaving class 5 of the elementary school with excellent results, attended the grammar-school in Arad. His first teacher in natural history was the botanist GÁBOR PERLAKY, who encouraged his habit of collecting plants. His later teacher, ISTVÁN KRICSFALUSI, already employed him in the laboratory of natural history as a famulus.

After finishing class 5, he had to leave the grammar-school, primarily because of his political attitude. He then went to the teachers' training-school in Arad, where his teacher of natural history was the outstanding botanist Dr. JÁNOS WAGNER.

During the four years (1906—1910) spent in the teachers' training-school in Arad, he was sent by botanist Dr. JÁNOS WAGNER on several occasions for long study-trips in various regions of the country: Retyezát, the Máramaros Mountains, the lower reaches of the Danube, and the environment of the Crotain Plitvice Lakes. He participated in the student-tourist competitions, by describing these trips. He was honourably mentioned for his papers on some occasions. During his 4th year, his paper "An excursion to Lake Balaton" was published in the year-book (1910) for the teachers training-school. At the same time, he became champion in shot-putting, javelin and discus-throwing at the National Junior Athletic Championships in Budapest.

From the teachers' training-school in Arad he was awarded a teacher's diploma (summa cum laude) for elementary schools. He next went to Budapest to study in the Higher Elementary School-Teachers' Training College, where the renowned botanist GUSZTÁV MOESZ was his teacher. In his first semester he became professor's assistant and continued his diligent collecting in various regions of the country. With his paper "Diatoms of the Surian mountain lakes" he won the KÁROLY SCHILBERSZKY Millennium Prize of the Society of Natural Sciences. Besides his scientific work, he continued his successful sporting activity. In 1912, he wrote a monograph "A short survey and the present state of phylogenetical theories". Ever since that time, he has dealt intensively with phylogenetic and genetic problems.

In 1913, he was awarded the higher elementary-school teacher's and physical instructor's diploma (summa cum laude), and was next admitted to the University of Budapest. In 1914 he became a soldier but, as far as possible, continued to deal with his studies. In 1916, he became assistant to Professor WIECHOWSKY in the Pharmacological Institute of the University of Prague, and in the meantime studied at the Medical Faculty of the University as well.

In 1917, because of his state of health, he was exempted from military service, and appointed assistant teacher in Csáktornya.

In Prague he continued dealing with considerations of evolution, and particularly with the evolutionary history of the vegetable kingdom. Here he wrote his paper "Ein Gedanke zur polyphyletischen Entwicklung der Pflanzenwelt" (1918).

In 1919, he was appointed to the Teachers' Training-Shool in Budapest, and then to the Higher Elementary-school Teachers' Training College where he worked until 1928. During this period, he wrote about fifteen text-books, approximately forty educational articles, and numerous scientific papers. These covered the whole field of botany.

In 1927, on the basis of his monograph "Sexuality of Cormophytes" he was appointed honorary lecturer in Budapest. The University doctor's degree was conferred to him as early as 1916.

In 1927, he was appointed to lecture botany and organize the Department in the University of Debrecen.

In 1928, the Higher Elementary-School Teachers' Training College from Budapest moved to Szeged and he was appointed to the Chair of Botany.

In 1933, his popular scientific work "The wonderful life of plants", prefaced by FERENC MÓRA, was published, followed in 1935 by his text-book "Introduction to genetics". His first xylotomic paper, entitled "Charcoal remains of the first lowland palaeolithic find", came out in connection with Ferenc Móra's excavations in 1936. In 1939 he demonstrated that in the Magdalenian Period there were living firs and cembra pines in the environment of Szeged. Then in his paper "Pollen-analytical investigations of the mammoth find at Öthalom" (1940) he described, on the basis of spores and pollens, the underwood of these forests as well. This was the first palinological monograph in Hungary to be illustrated with drawings.

In the meantime, while determining several prehistoric wood remains, he realized how difficult it is to identify these. In 1938, as a first trial, he wrote his paper "The determining key to the indigenous deciduous trees in this country on a histological basis". Since then he has dealt more and more with xylotomic investigations. His book "The Identification of Central-European Dicotyledonous Trees and Shrubs Based on Xylotomy" was published in 1945.

This book aroused great interest both in Hungary and abroad, and more and more palaeontologists have turned to him to determine fossilized and carbonized wood remains from Poland, U.S.S.R., Germay, Belgium etc.).

In the determinations, the identification of Pinaceae was particularly problematical because of the lack of any comparative material. He therefore began to deal intensively with the xylotomy of the living Conifers. This was the subject of his academic doctoral dissertation, made in 1955. (Identification of living Gymnosperms on the basis of xylotomy.) This work was later published, and is considered a fundamental text-book all over the world. In Hungary and at the International Book Display in Leipzig it was awarded the title" The most beautiful book of the year".

In 1959 his magnum opus in the domain of the anatomy of European deciduous trees was published in German (Holzanatomie der europäischen Laubhölzer und Sträucher), and received great appreciation. The work containing his original theory was published in a revised form in Hungarian and English, under the title "The phylogeny of sexuality and triphyletic evolution of the landplants" and attracted great attention all over the world.

In 1965 he retired at the age of senventy-five, but has continued to work with unflagging enthusiasm. New monographs were published in rapid succession: in 1967 "Fossil Gymnosperm Woods in Hungary from the Permian to the Pliocene", ion 1968 "Xylotomy of the living Cycads" and "Einführung in die Paläoxylotomie", and in 1972 "Xylotomy of the living Conifers". Besides these monographs, he has published many scientific papers in Hungarian and foreign journals.

The wide interests of Professor GREGUSS have included several branches of biological sciences, where he has produced papers of permanent value. For example, he has written 5 papers on natural philosophy (Unity of Nature), 12 on plant physiology, 15 on external morphology, 41 on taxonomy, 3 on plant geography, 12 on phylogenetics, 18 on evolutionary history, 10 on genetics, 58 on palaeontology, 5 on palinology, and 8 on ecology.

The number of his school-books, University text-books and lecture notes are 36. He has performed considerable popularizing work, as well, his papers of such a character numbering 25. The overall number of his works exceeds 300, 25 of these being books.

Professor GREGUSS deservedly enjoys wide-ranging appreciation. In 1956, he obtained the Academic doctor's degree in biological sciences. The Government of the Hungarian People's Republic decorated him with the Gold Order of Labour in 1955, 1959, and 1965, with the Silver Kossuth-Prize in 1958. On two occasions he was Vice-Dean, on one occasion Dean of the Faculty of Science, and on one occasion Rector of the University of Szeged.

He is a honorary member of several Hungarian and foreign scientific societies (International Union of Dendrology, International Society of Taxonomy, International Society of Palaeontology, International Society of Wood-anatomists, International Society of Morphology, German Society of Botany, etc), At present, heis maintaing wide-ranging scientific relations with more than 600 researchers. Hungarian and foreign specialists have named several living or fossil plants after Professor Greguss.

Besides his very considerable scientific and educational activity, Professor GREGUSS has organized the Department of Botany of the University of Szeged and for twenty-five years has reconstructed the Botanical Garden and developed it to high international renown. Many of his several hundred pupils are University and College professors, senior lecturers, Academic Doctors or Candidates in the biological sciences.

DR. IMRE HORVÁTH

Acta Biologica Szeged. *21* (1—4), pp. 9—20 (1975)

# COMPARATIVE WEED-INVESTIGATIONS IN TRADITIONALLY-CULTIVATED AND CHEMICALLY-TREATED WHEAT AND MAIZE CROPS. IV. STUDY OF THE WEED-SEED CONTENTS OF THE SOILS OF MAIZE CROPS

RÓZSA FEKETE

*Department of Botany, Attila József University, Szeged*

(Received June 15, 1974)

## Abstract

Weed-seed content investigation of the soils of maize plots of four farms, cultivated traditionally or treated with Hungazin PK for various periods and under the first- or second-year after-effect, led to the collowing findings:

*a* The weed-seed reserves of the 0—40 cm layers of the soils examined decreased very considerably during 10 years of traditional cultivation. A decisive role is played in the improvement of the soils by the more modern large-scale agrotechnology.

*b* The effect of the aminotriazines leading to a decrease in the weed-seed content depends appreciably on the type of the soil.

On Mezőség soils, compared to the traditionally-cultivated areas the weed-seeed reserves did not decrease, or did so to only a small extent ,as a result of chemical treatment for 1—3 years. This is understable, because of the slow and protracted germination of the majority of the weed seeds. In addition, the partial recontamination with weed seeds occurs on these soils, since the herbicide does not ensure complete elimination of the weeds.

The effect of the use of aminotriazines on reducing the weed-seed reserves is manifested better and more quickly on the lighter soils. In accoradance with this, a decrease of about 30—35 p. c. in the weed-seed contamination can be observed on sandy adobe and sandy soils following chemical exspraying for several years.

*c* As regards the overall evaluation of the data relating to soils of crops under Hungazin after-effect, it can be said that chemical treatment on one occasion has no, or only a very slight positive effect on the development of the weed-seed content in the following year. On areas under such an after-effect, therefore, the decrease of the weed-seed contamination is not to be expected; indeed, an increase can be reckoned with, particularly if *Echinochola crus-galli* and the panic grasses are predominant.

As regards protection against weeds, it is extremely important to know not only the weed cover, but also the weed-seed content of soils. The most significant surveys of the weed-seed reserves of agriculturally-cultivated soils in Hungary have been carried out by BENCZE (1954, 1958). His investigations revealed that as a consequence of the incorrect agrotechnology soils in Hungary were saturated with weed seeds: in the upper 0—20 cm soil layers he found 33,000—241,000 weed seeds per square metre, this concentration being much in excess of equivalent data published abroad (KORSMO, 1930; WEHSARG, 1954).

With one exception (the study by MÁTHÉ—PRÉCSÉNYI (1968) in 1967), Hungarian weed researches have been characterized by the fact that the weed covers of the crops and the weed-seed reserves of the soils have been examined completely independently of one another, whereas in the interest of the correct organization of protection against weeds it would be desirable to examine the two together.

During weed coenological surveys in 1961, the present author also carried out simultaneous examinations of the weed-seed contents, but the results were not published at that time. This research was continued in 1963, and for several years extended not only to the traditional cultivation, but also to the changes in the weed-seed reserves of maize soils as a result of large-scale agrotechnology. In addition, studies were also made of the effects of the very intensively active Hungazin on the weed-seed reserves of the soils, for in this respect concrete research results were not available in either the Hungarian or the foreign literature up to the beginning of the experiments (1963).

The present paper contains the results of the examinations in 1963; these were prepared as part of a doctoral dissertation in 1967, but have not yet been published. Similarly to earlier papers dealing with weed coenology, this work was done at Vácrátót.

## Investigation sites and methods

Since examinations of weed-seed contents demand very much work and time, the surveys were not carried out on every farm where coenological recordings were made, but only on those which seemed suitable as constant investigation sites for a prolonged period, or where in part comparison too was possible (e.g. Mezőhék). The examinations of the weed-seed reserves extended to the soils of maize crops on the State Farms at Mezőnagymihály, Mezőhék, Enying and Lábod; these crops had been cultivated traditionally, had been treated for 1—3 years with Hungazin PK (Atrazin), and were in the first or second year of the after-effect. From every area examined on each of these farms, 10 samples were taken at each depth, from depths ranging from 0 to 40 cm, at 10 cm interavals, i.e. a total of 40 samples each from every treatment, and all of these samples were analyzed. Examinations to such depths appeared necessary bacause, with the increase of the mechanization, deeper soil cultivation is applied increasingly more often. The borer used to take samples had a base area of 19.625 $cm^2$. The soil samples were taken everwhere on the second weed survey of the maize crops (in the second half of August and the first week of September).

In the examinations of the weed-seed reserves of the soils the $ZnCl_2$ solution separation method used by BENCZE (1954, 1958) was employed.

The degree of weed-seed contamination is given per square metre. Average values for each species were calculated from the results, for the various soils (Mezőnagymihály, Mezőhék, Enying), treatments and depths, and are tabulated for 25 weed species: in general those from which more than 100 seeds per square metre were found from 0 to 40 cm. The results for the Lábod State Farm are given separately (Table 4), because of the adobe or partially sandy soil. The numbers of seeds per square metre for the late-summer annuals ($T_4$) are given at the foot of the Tables for every treatment and depth, since these occur in the greatest amount from all the life-form groups; also given are the numbers of seeds of the total annuals, and the perennials (H+G), and the numbers of species of weed seeds found (Tables 1—4). (For every treatment the total number of weed seeds is also discussed in the text; in the majority of cases this is the sum of the total T and H+G.)

The weed seeds were determined on the basis of the handbook of UJVÁROSI (1957), the weed-seed collections of the present author and of Ujvárosi, and the handbook of BRONWER—STÖHLIN (1955). Those weed seeds which could not be identified on this basis were identified by means of the seed collection of the Országos Vetőmag Felügyelőség (National Seed Inspectorate), to whom the author expresses her grateful thanks.

The agrotechnological data relating to the investigation sites, and the details of the chemical treatments applied, are reported in the papers containing the weed-coenological examination results obtained from the same sites (FEKETE, 1964; published 1974a, b).

## Results and discussion

### A. Examinations of weed-seed reserves on Mezőség soils

#### 1. Weed-seed reserves of traditionally-cultivated maize soils

The most frequent weed seeds in traditionally-cultivated maize crops on Mezőség soils are given in the first part of Table 1, referred to 1 m². The examinations indicated that the number of weed seeds in the 0—40 cm traditional maize soils of the three State Farms (Mezőnagymihály, Mezőhék, Enying) varied between 5,503 and 14,908, and the average weed-seed contamination was 10,932 per m². A study of the distribution of the weed seeds according to depth showed that in all sites the 10—20 cm level of the soils contained the most weed seeds (2,089—5,841, and on average 4,240 per m²). As can be seen from Table 1, the 0—10 and 20—30 cm layers exhibit roughly the same degree of contamination (2,955 and 2,870, respectively) on average for the three investigation sites, while the fewest weed seeds are to be found in the 30—40 cm layer (866 per m²). Naturally there were various differences between the individual farms: the highest weed-seed contamination occurred in the Mezőnagymihály soils (14,908), and the lowest in those at Mezőhék (5,503). From the data at the foot of the Table it can be seen that the seeds of the late-summer annuals (T ) are found in the greatest amount in the soils (mainly *Stachys annua, Chenopodium album, Amaranthus retroflexus* and *Echinochloa crus-galli)*, and in comparison with these the other life-form groups are insignificant. This is understandable, since the data of the coenological surveys too indicate that the T life-form, and to a smaller extent the perennial root-like couch-grasses ($G_3$), predominate in cultivated land in Hungary (FEKETE, 1963, 1964, published 1974; UJVÁROSI, 1966). The tremendous seed yield of the former is well-known, while it is also known of the latter that primarily vegetative multiplication is decisive in their group, although the seed is also ripened on areas treated with aminotriazine (second half of Tables 1 and 2).

If the results obtained are compared with the weed-seed examination data of BENCZE (1954), obtained in part from the same sites and in part from different sites, but similarly in Mezőség soils, it is observed that compared to the earlier weed-seed concentrations of 33,596—54,094 per m² found in 1952 (the year immediately following the reorganization) in the 0—20 cm levels at Iregszemcse, Pusztapó and Bánkút, there was a significant improvement of the soils in the following 10 years. Thus, if the same levels (0—20 cm) are considered, it can be stated that at present the weed-seed reserves of the soils are only a quarter, or less, compared to the earlier values. This positive change is particularly striking in the case of Mezőhék—Kétpó (earlier known as Pusztapó), where the previous contamination 33,596 per m² for 0—20 cm (BENCZE, 1954) has decreased by a factor of more than ten (3,006 for the same level). At this latter site a large role in the improvement of the soils has been payed by the correct agrotechnology applied on the farm (UNGÁR, 1959), which is otherwise well reflected by the data of weed-coenological surveys here (FEKETE, 1964, published 1974a, b).

### 2. Weed-seed reserves of first-year Hungazin-treated maize soils

On average for the three farms, the first-year Hungazin-treated maize soils containe about 70 per cent more weed seeds (18,548 per $m^2$) than those cultivated traditionally (second half of Table 1) (10,932 per $m^2$). In corresponding soils at Mezőnagymihály about three times as many seeds (32,533 per $m^2$) were found as in the traditionally-cultivated soils; at Enying and Mezőhék increases of about 40 per cent and 10 per cent, respectively, were observed compared to the control. Naturally, this did noe occur as a result of the chemical treatment, since up to the time of sampling (the second survey of the maize crops, in the second half of August and the beginning of September in 1963) the weed-seed yield for that year had not yet entered the soil, or only in part, either in the traditionally-cultivated, or in the chemically-treated areas. The large differences in the weed-seed contents in the individual investigation rates can be explained by the different plant-pyrdicotions and soil-operations, and by the unsatisfactory care of the plants. (Thus, because of the presence of the hybrid-section in this farm-unit of the Mezőnagymihály State Farm, maize is almost always grown on these plots, so far in the traditional way, or at least with a herbicide not possessing such a lasting action as that of Hungazin PK. The hoeing monocultures favour the multiplication of just the late-summer species ($T_4$). On the first-year sprayed maize area at Enying lucerne had been grown for the previous three years, and this is why it was more contaminated.)

From an examination of the distribution of the weed-seeds according to depth it can be stated that the excess compared to the traditional soils is contained primarily in the uppermost two layers, and here too, compared to the control, the 10—20 cm layer is the most contaminated (second half of Table 1).

Analysis according to life-form indicates that the predominant seeds in the first-year Hungazin-treated maize soils too are those of the late-summer species ($T_4$), mainly *Amaranthus retroflexus, Chenopodium album* and *Stachys annua.*

### 3. Weed-seed reserves of maize soils treated with Hungazin for several years

Mezőség soil areas systematically treated with Hungazin or Atrazin for several years were examined only at Enying. As already reported in the coenological studies (FEKETE, 1964, published 1974a, b), in this farm all of the chemically-treated maizes were hoed, the number of hoeings on these plots being exactly the same as on those cultivated traditionally. Accordingly, apart from the herbicide effect, the ecological conditions of germination of the weed seeds were the same for all of the maize crops on the farm during the growing period. Although the aminotriazine agents do not act directly on the germination of the seeds, they can affect it indirectly as superselective herbicides.

If the results of weed-seed investigations on the plots of this farm which had been treated chemically are considered, it is surprising that their soils contain almost the same weed-seeds as those of traditionally-cultivated crops, and therefore these results are not given separetely. In crops treated chemically for 3 years a decrease of only 15 p. c. was observed (Table 2). Comparison of the contaminations of the

*Table 1.* More frequent weed seeds in maize crops on Mezőség soils (number per m²) (overall data)

| Treatment | Traditionally cultivatet | | | | | Hungazin (first-year treatment) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Depth (cm) | 0—10 | 10—20 | 20—30 | 30—40 | 0—40 | 0—10 | 10—20 | 20—30 | 30—40 | 0—40 |
| *Trifolium arvense* | 33.9 | 50.9 | 67.9 | 17.0 | 169.7 | 76.9 | 91.1 | 82.3 | 47.0 | 297.6 |
| *Hibiscus trionum* | 67.9 | 254.7 | 288.7 | 33.9 | 645.2 | 81.0 | 101.9 | 67.9 | 33.9 | 284.7 |
| *Convolvulus arvensis* | 33.9 | | 50.9 | 33.9 | 118.7 | | 26.1 | 60.1 | 17.0 | 103.2 |
| *Heliotropium europaeum* | 33.9 | | | | 33.9 | 101.9 | 13.1 | | 17.0 | 132.0 |
| *Ajuga chamaepitys* | 135.8 | 101.8 | 67.9 | | 305.5 | 118.9 | 73.6 | 101.9 | 50.9 | 345.3 |
| *Stachys annua* | 305.7 | 526.5 | 305.7 | 254.8 | 1392.5 | 286.1 | 762.8 | 469.0 | 365.7 | 1883.6 |
| *Veronica triphyllos* | | | | | | 78.4 | 209.0 | 145.7 | | 433.1 |
| *V. hederifolia* | 17.0 | 17.0 | | | 34.0 | 163.3 | 256.9 | 90.1 | | 510.3 |
| *Plantago major* | | 17.0 | 17.0 | | 34.0 | 84.9 | 113.2 | 135.8 | 50.9 | 384.8 |
| *Sinapis arvensis* | 50.9 | 50.9 | 17.0 | 33.9 | 152.7 | 50.9 | 50.9 | 43.1 | 111.0 | 255.9 |
| *Diplotaxis muralis* | 17.0 | 67.9 | 33.9 | | 118.8 | 17.0 | 37.7 | 67.9 | 17.0 | 139.6 |
| *Capsella bursa-pastoris* | 17.0 | 17.0 | | | 34.0 | 33.9 | 80.8 | 47.0 | | 161.7 |
| *Ambrosia elatior* | 67.9 | 50.9 | 17.0 | 17.0 | 152.8 | 101.9 | 150.9 | 101.9 | | 354.7 |
| *Arenaria serpyllifolia* | 169.8 | 305.7 | 220.8 | 67.9 | 764.2 | 50.9 | 226.5 | 50.9 | 33.9 | 211.2 |
| *Chenopodium album* | 475.6 | 645.4 | 237.7 | 84.9 | 1443.6 | 330.5 | 700.6 | 471.6 | 98.0 | 1600.7 |
| *Amaratnhus retroflexus* | 288.7 | 475.8 | 169.8 | 34.0 | 968.3 | 2799.9 | 3509.3 | 670.2 | 52.2 | 7031.6 |
| *A. albus* | 17.0 | 34.0 | 51.0 | | 102.0 | 143.7 | 199.9 | 65.3 | 13.1 | 422.0 |
| *Anagallis arvensis* | 17.0 | 186.8 | 118.9 | 1 7.0 | 339.7 | 101.9 | 188.7 | 301.8 | 101.9 | 694.3 |
| *Polygonum lapathifolium* | 17.0 | 33.9 | 33.9 | 33.9 | 118.7 | | 50.9 | 30.0 | | 80.9 |
| *P. aviculare* | 50.9 | 33.9 | 17.0 | 34.0 | 135.8 | 43.1 | 31.9 | | | 75.0 |
| *Bilderdykia convolvulus* | 101.9 | 50.9 | 67.9 | 34.0 | 254.7 | 98.0 | 126.3 | 118.9 | | 343.2 |
| *Digitaria sanquinalis* | 220.8 | 84.9 | 50.9 | | 356.6 | | | | | |
| *Echinochloa crus-galli* | 237.8 | 407.8 | 458.6 | 84.9 | 1189.1 | 582.7 | 323.8 | 26.1 | 95.4 | 1028.0 |
| *Setaria glauca* | 169.8 | 220.8 | 236.6 | | 627.2 | 52.3 | 121.5 | 90.1 | 13.0 | 277.0 |
| *S. viridis* | 152,8 | 305.7 | 220.8 | 17.0 | 696.3 | 167.2 | 198.4 | 120.2 | 47.0 | 532.8 |
| Total late-summer species ($T_4$) | 2476.8 | 3662.6 | 2513.0 | 730.5 | 9382.9 | 5155.7 | 6851.1 | 2543.9 | 965.6 | 15516.3 |
| Total annuals (T) | 2870.7 | 4155.3 | 2801.7 | 832.4 | 10 660.1 | 5567.3 | 7653.8 | 3318.8 | 1101.5 | 17 641.4 |
| Total perennials (H, G) | 84.9 | 84.9 | 84.9 | 33.9 | 288.6 | 219.7 | 328.0 | 290.0 | 81.0 | 931.9 |
| Number of weed species | 36 | 34 | 28 | 18 | 44 | 31 | 38 | 34 | 20 | 47 |

*Table 2.* More frequent weed seeds )per m²) in maize soils on the Enying State Farm

| Treatment | Traditionally cultivated | | | | | Treated with Atrazin for 3 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Depth (cm) | 0—10 | 10—20 | 20—30 | 30—40 | 0—40 | 0—10 | 10—20 | 20—30 | 30—40 | 0—40 |
| *Trifolium arvense* | 101.9 | | 101.9 | 51.0 | 254.8 | | | | | |
| *Hibiscus trionum* | 51.0 | 51.0 | | 51.0 | 153.0 | 101.9 | | 51.0 | | 152.9 |
| *Euphorbia falcata* | 51.0 | 101.9 | 51.0 | 51.0 | 254.8 | 101.9 | | 51.0 | | 152.9 |
| *Convolvulus arvensis* | | | | | | 101.9 | 101.9 | | | 203.8 |
| *Heliotropium europaeum* | 51.0 | 51.0 | | | 102.0 | 152.9 | 203.8 | | 51.0 | 407.7 |
| *Verbena officinalis* | 51.0 | 51.0 | | | 102.0 | | | | 51.0 | 51.0 |
| *Ajuga chamaepitys* | 253.7 | 203.9 | 152.9 | | 611.5 | 305.7 | 254.8 | 101.9 | 101.9 | 764.3 |
| *Stachys annua* | 713.4 | 713.4 | 611.5 | 509.6 | 2547.8 | 305.7 | 407.6 | 152.9 | | 866.2 |
| *Veronica hederifolia* | 51.0 | 51.0 | | | 102.0 | | | 101.9 | | 101.9 |
| *Plantago major* | | 51.0 | | | 51.0 | | | | 152.9 | 152.9 |
| *Diplotayis muralis* | | 203.8 | | | 203.8 | | 51.0 | 101.9 | | 152.9 |
| *Camelina microcarpa* | 101.9 | | 51.0 | | 152.9 | | 51.0 | | | 51.0 |
| *Ambrosia elatior* | 203.8 | 152.9 | 51.0 | 51.0 | 458.7 | 356.6 | 407.6 | 305.7 | | 1070.1 |
| *Arenaria serpyllifolia* | 509.6 | 917.2 | 662.4 | 203.8 | 2293.0 | | 101.9 | 152.9 | | 254.8 |
| *Chenopodium album* | 815.3 | 764.3 | 305.7 | 101.9 | 1987.1 | 866.2 | 764.3 | 509.6 | 152.9 | 2293.0 |
| *Amaranthus retroflexus* | 152.9 | 203.9 | 51.0 | 51.0 | 458.8 | 152.9 | 354.8 | 254.8 | | 663.4 |
| *Anagallis arvensis* | | 509.6 | 305.7 | | 815.3 | 51.0 | 305.7 | 356.6 | 101.9 | 815.2 |
| *Polygonum lapathifolium* | 51.0 | 51.0 | 51.0 | | 153.0 | 101.9 | | 101.9 | 51.0 | 254.8 |
| *Bilderdykia convolvulus* | 203.8 | 101.9 | 51.0 | 51.0 | 407.7 | | 101.9 | | | 101.9 |
| *Sorgum halepense* | | | | | | 51.0 | | 51.0 | | 102.0 |
| *Agropyron repens* | 101.9 | 152.9 | 51.0 | | 305.8 | 51.0 | | | | 51.0 |
| *Eragrostis poaeoides* | 51.0 | 51.0 | | | 102.0 | | | | | |
| *Echinochloa crus-galli* | | | | | | 101.9 | 203.8 | 152.9 | | 458.6 |
| *Setaria glauca* | | | | | | | 101.9 | 51.0 | | 152.9 |
| *S. viridis* | | 51.0 | | 51.0 | 102.0 | 203.8 | 152.9 | 101.9 | | 713.4 |
| Total late-summer species (T4) | 2802.7 | 3363.5 | 1834.7 | 1019.5 | 9020.4 | 2904.4 | 3260.0 | 2294.0 | 560.6 | 9019.0 |
| Total annuals (T) | 3618.2 | 4484.6 | 2548.1 | 1223.3 | 11874.2 | 2955.4 | 3412.9 | 2650.7 | 560.6 | 9579.6 |
| Total perennials (H, G) | 152.9 | 254.9 | 51.0 | | 458.8 | 305.9 | 254.8 | 51.0 | 203.9 | 611.5 |
| Number of weed species | 19 | 23 | 15 | 10 | 30 | 19 | 20 | 17 | 7 | 34 |

individual soil levels did not reveal an essential difference either; thus, the positive effect of chemical treatment for 2 or 3 years develops more slowly on the Mezőség soil than on lighter soils, for instance, as will be seen later. The resistance of the weed-seeds was proved by the fact that the soil could be shown to contain the seeds of weed species which had certainly not occurred since chemical treatment (as demonstrated by the coenological surveys) in crops on areas treated with the same dose of aminotriazine. A decisive role in the slow purification of the soils from the weed seeds is played by the resting state of the seeds, which can be induced by several factors (PETERSEN, 1951; WEHSARG, 1954; CORCKER—BARTON, 1957; THURSTON, 1960; NÍKOLAYEVA, 1967). In some of the weed seeds (e.g. *Convolvulus arvensis, Hibiscus trionum)* the resting state is brought about by the "hard covering" of the seeds (SRIPLENG—SMIITH, 1960; CZIMBER—REISINGER, 1968). Since such hard-skinned seeds are in dormancy they resist chemical herbicides and again give rise to weed cover on germinating after cessation of the herbicide effect, as shown by the data of late-summer (II) weed surveys on maize crops under the after-effect of aminotriazine (FEKETE, 1964; published 1974a, b). In addition to what has already been said, another cause of the slow purification of the soils may be that partial recontamination with weed seeds occurs on areas treated with chloraminotriazine, for this herbicide does not ensure total elimination of weeds.

### 4. Weed-seed contents of maize soils under after-effect of Hungazin PK

It was mentioned above that the tratment of the maize plots under the after-effect at Enying and Mezőhék was exactly the same as that on those cultivated traditionally there, apart from the herbicide. At Mezőnagymihály the crops were hoed mechanically twice and by hand once, in contrast with the maize cultivated traditionally, where second serial hoeing too was carried out.

From the overall data from the investigations on areas under first-year Hungazin after-effect, the 0—40 cm cultivated layer contains 12,548 weed seeds per $m^2$; the average is thus about 10—12 p. c. worse than for the traditionally-cultivated areas. If the average results are broken down for the individual farms, it can be established that the weed-seed content in the soils of the Enying plots has decreased a little compared to the control. At Mezőnagymihály and Mezőhék, on the other hand, an increase can be observed. The overwhelming proportion of the weed-seed increase is contained in the 10—20 cm layer (first part of Table 3).

As reported in the paper dealing with the results of the weed coenological investigations (FEKETE, 1964, published 1974a, b), a huge weed-mass, mainly *Echinochloa* and Setaria, lived in places on the areas under the first-year Hungazin after-effect (including Mezőnagymihály too). Up to the time of the sampling, however, these had only partially shed their seeds. The conciderable seed-rotation occurred in the period after the survey and the sampling, and could be demonstrated only in samples of soil taken in the following year (1964). But even then not entirely, for an appreciable part of the weed seeds entering the soil (mainly *Echinichloa cruss-galli* and panic grasses) germinated during the growing time, as proved by the coenological surveys in 1964. Weed seed germination experiments from 1961 also confirm that *Echinochloa crus-galli* and the panic grasses *(Setaria viridis, S. glauca)* had already shed their seeds at the end of summer; these entered the soil and germinated in a fairly high proportion in the following year.

Weed-seed contents of plots under second-year Hungazin after-effect were studied only at Mezőhék, and these results are given in the second half of Table 3. These data are almost the same as those of the control there.

## B. Weed-seed content investigations on sandy adobe — sandy soil

The Lábod State Farm is situated on sandy adobe, and partly on sandy soil. Here examinations were made on the weed-seed reserves of soils of maize crops cultivated traditionally or treated with aminotriazine for three years. These examinations revealed the very extensive contamination of the soil on this farm (Table 4). The Table contains weed-seed data on only 25 species, but of the seeds of the 51 weed species found in these two types of soil sample only three did not attain a concentration of 100 per $m^2$.

From the soil of maize cultivated traditionally 176,878 weed seeds were found per $m^2$ from the 0—40 cm layers, the bulk of which were late-summer annuals ($T_4$) (142,895 per $m^2$). BENCZE (1958) demonstrated such a huge weed-seed contamination only on the sandy soils at Nyíregyháza, where he counted 110,250—241,375 weed seeds per $m^2$ in the 0—20 cm layers in the various hoeing cultures. (In the same layers at Lábod there were 117,715 seeds per $m^2$.)

This extremely extensive contamination of the traditional maize soils at Lábod is attributed to a certain degree to the fact that on the occasion of the national weed coenological survey in 1949—50 this was the most densely weed-populated area of the country, and such a state cannot be changed from one day to another. In addition, another fact which undoubtedly contributed to such a mass presence of late-summer seeds, however, was that only maize has been grown on this area since 1961, and it is well known that the main multiplication sites of the late-summer varieties are monoculture maize crops.

Examination of the distribution of the weed seeds according to depth reveals that they progressively decrease in number on passing downwards from the surface, but there is no appreciable difference between the contaminations of the individual soil layers from 0 to 30 cm. In the 30—40 cm layer, however, the number of weed seeds exhibits a sudden considerable decrease (first part of Table 4).

The number of weed seeds in soil of maize which had been treated chemically systematically for three years was about 30—35% lower than in the tradition-cultivated areas serving as control, but even then the number was very high: 112,153 per $m^2$. 75—80% of these weed seeds similarly belonged to the late-summer annual life-form ($T^2$).

If the weed-seed reserves of the individual soil levels are compared with those for traditionally-cultivated maize, it is observed that purification from the weed seeds as a result of the 3 years' chemical treatment occured over the total depth of soil examined. Although the number of weed seeds, including the late-summer species too, decreased, attention must be drawn to an unfavourable phenomenon. In these areas not only the coverage of *Echinochloa crus-galli* increasedt but also the number of its seeds in the soil. At the same time it is encouraging that during this time the seeds of *Amaranthus retroflexus* and *Chenopodium album* practically disappeared from the soil, as is otherwise confirmed by the data of germination experiments with the seeds of these latter two species in sandy soil.

*Table 3.* More frequent weed seeds (per m²) in Mezőség maize soils under Hungazin after-effect (overall data)

| Treatment | Areas under first-year Hungazin after-effect | | | | | *Areas under second-yer aminotrizaine after-effect | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Depth (cm) | 0—10 | 10—20 | 20—30 | 30—40 | 0—40 | 0—10 | 10—20 | 20—30 | 30—40 | 0—40 |
| *Trifolium arvense* | 38.2 | 101.9 | 63.7 | 12.7 | 216.5 | | | | | |
| *Malva neglecta* | 25.4 | 76.4 | 76.4 | | 178.2 | | | | | |
| *Hibiscus trionum* | 38.2 | 38.2 | 76.4 | 25.4 | 178.2 | | 254.8 | 101.9 | 51.0 | 407.7 |
| *Convolvulus arvensis* | 76.4 | 114.6 | 50.9 | 25.5 | 267.4 | | | | 51.0 | 51.0 |
| *Heliotropium europaeum* | 25.4 | 51.4 | 12.7 | 50.9 | 178.7 | | | | | |
| *Ajuga chamaepitys* | 76.4 | 89.1 | 76.4 | 25.4 | 267.3 | | 101.9 | | 51.0 | 152.9 |
| *Stachys annua* | 356.2 | 853.5 | 496.8 | 178.4 | 1184.9 | 203.8 | 815.3 | 254.8 | 305.7 | 1579.6 |
| *Veronica hederifolia* | 12.7 | | | | 12.7 | 51.0 | | | 51.0 | 102.0 |
| *Fumaria schleicheri* | 12.7 | 63.7 | 12.7 | | 89.1 | 51.0 | 51.0 | | | 102.0 |
| *Sinapis arvensis* | | 25.4 | 12.7 | | 38.3 | 305.7 | 152.9 | 254.8 | 458.6 | 1172.0 |
| *Diplotaxis muralis* | | 12.7 | 63.7 | 38.2 | 114.6 | | | 51.0 | | 51.0 |
| *Thlaspi arvense* | | | 25.4 | | 25.4 | 51.0 | | 101.9 | 152.9 | 305.8 |
| *Capsella bursa-pastoris* | 24.5 | 63.7 | 25.4 | | 114.5 | | | | | |
| *Ambrosia elatior* | 76.4 | 89.1 | 76.4 | 5.90 | 292.8 | | | | | |
| *Arenaria serpyllifolia* | 25.4 | 50.9 | 38.2 | | 113.6 | | | | | |
| *Polycnemum arvense* | | 127.4 | 38.2 | 25.4 | 191.0 | | | | | |
| *Chenopodium album* | 394.9 | 700.6 | 726.1 | 229.3 | 2050.9 | 356.7 | 51.0 | | 407.6 | 815.3 |
| *Amaranthus retroflexus* | 76.4 | 165.7 | 114.6 | 50.9 | 407.6 | | 51.0 | | 51.0 | 102.0 |
| *A. albus* | | 50.9 | 50.9 | | 101.8 | | | | | |
| *Polygonum aviculare* | 50.9 | 89.1 | 76.4 | 12.7 | 229.1 | | | | | |
| *Bilderdykia convolvulus* | 24.5 | 152.8 | 216.5 | 25.5 | 419.3 | | | | | |
| *Digitaria sanquinalis* | 127.4 | 127.4 | 12.7 | | 267.5 | | | 152.9 | | 152.9 |
| *Echinochloa crus-galli* | 331.2 | 280.2 | 191.2 | 38.2 | 840.8 | 101.9 | 51.0 | 51.0 | 101.9 | 305.8 |
| *Setaria glauca* | 114.6 | 216.5 | 318.4 | 101.9 | 751.4 | 51.0 | 51.0 | | | 102.0 |
| *S. viridis* | 560.6 | 1184.7 | 560.5 | 420.4 | 2726.1 | 152.9 | 51.0 | 51.0 | 203.8 | 458.7 |
| Total late-summer species (T4) | 2344.0 | 4547.9 | 3324.9 | 1324.9 | 11541.7 | 917.3 | 1477.9 | 662.6 | 1223.0 | 4280.8 |
| Total annuals (T) | 2446.0 | 4879.2 | 3477.8 | 1363.1 | 12166.1 | 1376.0 | 1681.8 | 1019.3 | 1885.5 | 5962.6 |
| Total perennials (H, G) | 101.8 | 152.8 | 89.1 | 35.2 | 381.9 | | | | 51.0 | 51.0 |
| Number of weed species | 26 | 38 | 33 | 22 | 49 | 10 | 10 | 8 | 11 | 17 |

* Refers only to Mezőhék.

*Table 4.* More frequent weed seeds in the maize soils on the Lábod State Farm (number per m²)

| Treatment | Traditionally cultivated | | | | | Treated with Atrazin for 3 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Depth (cm) | 0–10 | 10–20 | 20–30 | 30–40 | 0–40 | 0–10 | 10–20 | 20–30 | 30–40 | 0–40 |
| *Ranunculus sardous* | 254.8 | 622.8 | 283.1 | | 1160.7 | 305.7 | 866.2 | 256.7 | 51.0 | 1579.6 |
| *Aphanes arvensis* | 636.9 | 452.9 | 736.0 | 169.9 | 1995.7 | 1019.1 | 407.6 | 305.7 | | 1732.4 |
| *Myosotys arvensis* | 2866.2 | 2321.3 | 4246.3 | 169.9 | 9603.7 | 866.2 | 305.7 | 407.6 | | 1579.5 |
| *Plantago major* | 700.6 | 396.3 | 169.9 | | 1266.8 | 611.5 | | | 51.0 | 662.5 |
| *Arabidopsis thaliana* | 1592.3 | 1472.0 | 2264.6 | 56.0 | 5385.5 | 866.2 | 152.9 | 560.5 | 51.0 | 1630.6 |
| *Viola arvensis* | 955.4 | 452.9 | 1981.6 | 113.2 | 3503.1 | | 51.0 | | | 51.0 |
| *Ambrosia elatior* | 828.0 | 1245.6 | 962.5 | | 3036.1 | 203.8 | 458.6 | 611.5 | 51.0 | 1324.9 |
| *Anthemis arvensis* | | | 396.3 | | 396.3 | | | | | |
| *Gypsophila muralis* | 3694.2 | 2094.8 | 2547.8 | 339.7 | 8676.5 | 1426.7 | 1273.9 | 1528.7 | 356.7 | 4586.0 |
| *Stellaria media* | 382.2 | 396.3 | 113.2 | 56.6 | 948.3 | | 51.0 | 203.8 | 51.0 | 305.8 |
| *Cerastium fontanum* | 254.8 | | 1358.8 | | 1613.6 | 407.6 | 51.0 | | | 458.6 |
| *Scleranthus annuus* | 1528.6 | 1188.9 | 1188.9 | 169.9 | 4076.4 | 1885.3 | 1228.7 | 713.4 | 51.0 | 4178.4 |
| *Spergularia rubra* | 63.7 | 56.6 | 226.5 | | 346.8 | 305.7 | | 152.9 | | 458.6 |
| *Herniaria hirsuta* | 573.2 | 906.9 | 566.1 | | 2045.2 | 254.8 | | 764.3 | 152.9 | 1172.0 |
| *Chenopodium album* | 509.6 | 226.5 | 226.5 | 56.6 | 1019.2 | 101.9 | | | | 101.9 |
| *Amaranthus retroflexus* | 254.8 | | | | 254.8 | 101.9 | | | | 101.9 |
| *Anagallis arvensis* | 636.9 | 339.7 | 905.9 | | 1882.5 | 254.88 | 101.9 | 356.7 | 51.0 | 764.4 |
| *Centenculus minimus* | 1273.9 | 849.3 | 679.4 | | 2802.6 | 866.2 | 1375.8 | 968.2 | 51.0 | 3261.2 |
| *Polygonum lapathifolium* | 509.6 | 452.9 | 396.3 | 56.6 | 1415.4 | 254.8 | 305.7 | 152.9 | 51.0 | 764.6 |
| *P. persicaria* | 191.1 | 452.9 | 226.5 | 113.2 | 983.7 | 152.9 | 611.5 | | | 764.4 |
| *P. aviculare* | 318.5 | 56.6 | 56.6 | | 431.7 | 866.2 | 254.8 | | | 1121.0 |
| *Juncus bufonius* | 42544.6 | 39176.5 | 31705.1 | 3679.9 | 117106.1 | 27057.1 | 24152.8 | 17936.2 | 3566.9 | 72713.0 |
| *Digitaria sanquinalis* | 191.1 | 56.6 | 169.9 | | 416.7 | 1579.6 | | 152.9 | | 1732.5 |
| *Echinochloa crus-galli* | 509.6 | 1132.3 | 1075.7 | 339.7 | 3057.3 | 2343.9 | 2140.1 | 4381.1 | 254.8 | 9120.9 |
| *Setaria glauca* | 445.9 | 339.7 | 452.9 | | 1238.5 | | | | | |
| Total late-summer species (T4) | 51861.9 | 47159.4 | 39688.1 | 4587.7 | 142895.1 | 35159.0 | 29605.0 | 26853.5 | 4535.3 | 96152.8 |
| Total annuals (T) | 61461.8 | 54632.6 | 53502.5 | 5321.8 | 174918.7 | 41783.0 | 33834.5 | 30471.4 | 4892.2 | 110879.2 |
| Species wintering at the soil level (H) | 828.0 | 736.0 | 283.1 | | 1847.1 | 815.3 | 51.0 | 51.0 | 102.0 | 1019.3 |
| Species witering in the soil (G) | | 56.6 | 56.6 | | 113.2 | 51.0 | 51.0 | 203.9 | 51.0 | 356.9 |
| Number of weed species | 28 | 29 | 33 | 12 | 41 | 30 | 23 | 25 | 17 | 42 |

The surprising resistance of the weed seeds is indicated by the fact that the seeds of many more weed species (altogether 51 from the two types of cultivated area) could be detected in the soil at Lábod than suggested by the data of the coenological surveys here. The seeds were found of species which were not observed at all in the course of the weed coenological surveys in 1963—1965. A few of these species (*Centaurea pannonica, Cerastium* fajolo, etc.) were contained in the 1949—50 weed list. At the same time a few weed species and their seeds were now detected sporadically which can be regarded as new data for the area (*Alopecurus myosuroides, Holcus, Galeopsis, Matricaria inodora* etc.).

## References


BENCZE, J. (1954): Iregszemcse, Pusztapó, Bánkút mezőségi talajainak gyommag-fertőzöttsége (Weed-seed contamination of the Mezőség soils of Iregszemcse, Pusztapó and Bánkút). — Agrártud. Egyetem Agronómiai Kar Kiadv. *1*, 3.

BENCZE, J. (1958): Szántóföldi gyommagvizsgálatok eredményei Nyíregyháza homoktalajain (Results of ploughland weed-seed investigations on the sandy soils of Nyíregyháza). — Agrártud. Egyetem Mezőgazd. Tud. Kar. Közl. 99—113.

BENCZE, J. (1969): A gyommagvak és termések csírázási feltételei (Germination conditions of weed-seeds and fruits). — Agrártud. Egyet. Mezőgazd. Tud. Kar Közl. 153—162.

BRONWER, W. — STÖHLIN, A. (1965): Handbuch der Samenkunde für Landwirtschaft, Gartenbau und Forstwirtschaft. — Frankfurt am Main.

CROCKER, W. —BARTON, L. W. (1957): Physiology of Seeds. — Waltham Marx. USA, Chr. Bot, Comp. p. 267.

CZIMBER, GY. — REISINGER, M. (1968): A *Convolvulus arvensis* kemény héjú magvainak a talajsterilizáló és gyökérherbicidekkel szembeni ellenállóképessége (Resistance of hard-skinned seeds of *Convolvulus arvensis* against soil-strelizing and root herbicides). — Növénytermelés *17*, 249—257.

FEKETE, R. (1964): Összehasonlító gyomvizsgálatok hagyományos művelésű és vegyszeresen kezelt búza- és kukoricavetésekben (Comparative weed investigations in traditinally-cultivated and chemically-treated wheat and maize crops.) — Manuscript, written in form of doctoral dissertation 1967, defended 1972.

FEKETE, R. (1964, published 1974a): Összehasonlító gyomvizsgálatok hagyományos művelésű és vegyszeresen kezelt búza- és kukoricavetésekben. II. A kukoricavetések gyomnövényzetében beállott változások (Comparative weed investigations im traditionally-cultivated and chemically-treated wheat and maize crops. II. Changes in the weed vegetation of maize crops). — Acta Biol. Szeged *20*. pp. 37—46

FEKETE, R. (1964, published 1974b): Összehasonlító gyomvizsgálatok hagyományos művelésű és vegyszeresen kezelt búza- és kukoricavetésekben. III. A gyomviszonyok alakulása Simazin, Atrazin (Hungazin PK) utóhatás alatt álló kukoricavetésekben és a talajok aminotriazintartalmának kimutatása (Comparative weed investigations in traditionally- cultivated and chemically-treated wheat and maize crops. III. Development of the weed conditions in maize crops under the after-effect of Simazin and Atrazin (Hungazin PK) and the detection of the aminotriazine contents of the soils). — Acta Biol. Szeged *20*.

HURLE, K. (1969): Der Einfluss langjähriger wiederholter Anwendung der Herbizide DNOC, 2,4-D, und MCPA auf ihren Abbau und der Unkrautsamen-Vorrat im Boden. — Diss. Inst. Pflanzanschutz. Univ. Hohenheim.

KEMENES, M. (1955): Beszámoló a hajdúszoboszlói tangazdaságban végzett vegyszeres gyomirtásró (Account of chemical herbicide treatment on the teaching-farm at Hajdúszoboszló.) — Növénytermelés *4*, 280—284.

KORSMO, E. (1930): Unkräuter im Ackerbau der Neuzeit — J. Springer Verlag, Berlin.

KOZMA, D. (1922): Gyommagvak a talajban (Weed seeds in the soil). — Kis. Közl. 25.

MÁTHÉ, I. — PRÉCSÉNYI, I. (1968): Adatok egy búzatábla fitomassza-produkciójához (Data on thel phytomass production of a wheat plot). — Agrártud. Közl. *3—4*, 253—256.

NIKOLAYEVA, M. G. (1967): Fiziologiya glubokava pokolya semyan. — Izd. "Nauka", Leningrad.
PETERSEN, A. (1951): Die Bekämpfung der Ackerungkräuter. — Akad. Verl.
SCHERMANN, Sz. (1966): Magismeret I—II (Seed-recognition, I—II). — Akadémiai Kiadó, Budapest.
SRIPLENG, A. — SMITH, F. H. (1960): Anatomy of the seed of Convolvulus arvensis. — Amer. Jour. Bot. Baltimore *47*, 386—392.
THURSTON, J. M. (1960): Dormancy in weed seeds. — Biol. Weeds. Blackweel Scientific Publications, Oxford, 69—82.
UBRIZSY, G. (1962): Vegyszeres gyomirtás (Chemical herbicide treatment.) — Budapest.
UJVÁROSI, M. (1957): Gyomnövények, gyomirtás (Eradication of weed plants.) — Budapest.
UJVÁROSI, M. (1966): A gyomnövényzet változása a szántóföldeken az elmúlt évtizedben (Change in the weed vegetation on ploughland during the past decade.) — MTA Agrártud. Oszt. Közl. *25*, 275—289.
UNGÁR, GY. (1959): Őszi búza termesztése a Héki Állami Gazdaságban (Production of winter wheat on the Hék State Farm.) — Magyar Mezőgazd. Budapest *14*, *9*.
WEHSARG, O. (1954): Ackerungkrauter. — Berlin.

Address of the author:
Dr. RÓZSA FEKETE
Department of Botany,
A. J. University, H—6701 Szeged,
P. O. Box 428, Hungary

Acta Biologica Szeged. *21* (1—4), pp. 21—34 (1975)

# LYCOPODIACEAE SPORES FROM LOWER CRETACEOUS DEPOSITS OF HUNGARY

M. JUHÁSZ

*Department of Botany, Attila József University, Szeged*

(Received November 30, 1974)

**Abstract**

The paper describes 21 species of Lycopodiaceae family from the Lower Cretaceous deposits of Transdanubian Central Mountains in Hungary. These 21 formspecies are recognised as belonging to five formgenera. They are following: *Retitriletes*, *Vadaszisporites*, *Foveosporites*, *Sestrosporites*, and *Camarozonosporites*. Four new combinations are proposed and two of the species described are new. The stratigraphic distribution of the Lycopodiaceae spores: most of the species of *Retitriletes*, *Sestrosporites* and *Foveosporites* described from the Neocomian, Aptian and Lower Albian strata. The younger sediments (Upper Albian—Lower Cenomanian) are characterized by the formspecies of *Vadaszisporites* and *Camarozonosporites*.

## Introduction

On Hungarian territory the Lower Cretaceous deposits can be found in the Transdanubian Central Mts and Villány Mts. Several works are dealing with the stratigraphic evaluation of these layers from which we emphasize Fülöp's papers (1958, 1964, 1966) as comprehensive works. The stratigraphic investigations are carried on in our days, as well.

In the course of the palynological investigation of the Transdanubian Lower Cretaceous deposits the comparatively uniform character of the flora of that period is conspicuous. The floras are characterized by the fossil representatives of Pteridophyte. About 60 per cent of the spores found are fern-spores, while the spores of the Lycopsida and Sphenopsida classes are present only in a lower number.

In our present paper, we are dealing, within the Lycopsida class, with the spores of some taxons of Lycopodiaceae family from the Lower Cretaceous.

## Previous Works

The histological, taxonomic, phytogeographical elaboration of the living Lycopodiaceae can be found in several monographs, textbooks, papers. The works of PRITZEL (in ENGLER—PRANTL, 1902), NESSEL (1939), KNOX (1950) and HARRIS (1955), reporting on about 420 to 450 Lycopodiaceae species, distinguish themselves even among these. These are included by a part of authors in two genera: *Lycopodium* L. and *Phylloglossum* KUNZE. Others, like HERTER (1909), emphasize subgenus *Urostachys* of genus *Lycopodium* and -classing 340 species or so therein — are

raising it to a rank of genus. NESSEL (1939) was already applying the following three genera: *Lycopodium* (L.) HERTER, *Phylloglossum* KUNZE, *Urostachys* (PRITZEL) HERTER. Our knowledge of the spores of the recent Lycopodiaceae is rather defective. The spores of about 25 per cent of the species described have been elaborated anyhow. Illustrations of spores are published by a number of authors, but the morphological grouping of spores is treated of first by KNOX (1950). He created five groups: three foveolate, one reticulate groups, and an echinate one. HARRIS (1955) revised Knox's work and the "Group Clavatum" is subdivided into two: a rugulate (hamulate) and a reticulate group. The classification of the recent Lycopodiaceae spores, that is best even today, is to be found in KRUTZSCH's publication (1963). He created four morphological groups, with twelwe morphotypes. These are the following:

1) foveolate spore-forms. Morphotypes: "selago", "phlegmaria", and "verticillatum".
2) reticulate spore-forms. Morphotypes: "paniculatum", "ramulosum", "sprucei", "annotinum", and "clavatum".
3) hamulate spore-forms. Morphotypes: "cernuum", "carolinianum", and "inundatum".
4) verrucate-echinate spore-form. Morphotype: "densum".

A great merit of KRUTZSCH's work is that it is comparing the recent morphotypes with the formgenera of the fossil dispersed spores. The author is proving that Retitriletes formgenus is a level corresponding to the "Clavatum" group, the *Camarozonosporites* to the "hamulate" spore-forms, and the Selagosporis to the "selago"-morphotype; therefore, the levels of recent and fossil organtaxons do not conform entirely to each other. But that doesn't exclude a relationship of Tertiary spore-forms, included in the above formgenera and in those described by him, to the recent Lycopodiaceae species. However similar some spore-forms may be to the recent ones, the Mesozoic palynological literature is treating the problem of botanical relationship very carefully. Some reference to the Lycopodiaceae-origin is only found at the most known forms of *Lycopodiumsporites austroclavatidites, clavatoides* . There are, anyway, several factors that incite us to try to recognize some relationship between the Lycopodiaceae species living today and the motherplant of the "lycopodiaceous" spores investigated by us. The main characteristics from among these are the following:

1) Phylogenetic connection
   All the recent Lycopodiaceae species have herbaceous stems. Among the fossil forms is the genus Lycopodites, which resemble in general form those of the extant Lycopodium. Lycopodites have been found from the Carboniferous to the present.
2) Anatomically the fossil *Lycopodites* are very similar to the living Lycopodiaceae; both genera have herbaceous, dichotomously branched stems, exarch protostele, microphyllous leaves, adaxial sporangia, and homosporous condition.

3) Geographical distribution
   Those still living are world-wide in distribution, but most species are found in the tropics.
   As PRITZEL (in ENGLER—PRANTL, 1902) is writing, their distribution has two focuses:

*a)* The Palaeotropic species were starting from South India, East-Himalaya towards Africa, Australia Polynesia, New-Zealand;

*b)* The Neotropic species diffused from the Brasilian and Columbian area of the Andes to other regions on the territory of South and Central America.

The Aptian—Albian stage of the Lower Cretaceous in the area of the European Flora Province is characterized by the breaking forth of the humid, subtropical-tropical climate, that may have facilitated the appearance of the Lycopodiaceae so rich in species.

4) Ecologic claim

The majority of recent Lycopodiaceae claim an equalized, humid atmosphere, they don't require too much heat and light. They are therefore the richest in species in mountains, in woods of not too thick vegetation, on islands and in places of oceanic climate. They generally prefer a substratum rich in organic matter (PRITZEL, in ENGLER PRANTL, 1902).

5) Their role in producing formations

It is equally characteristic both of the recent and Mesozoic Lycopodiaceae that they are not exerting any considerable influence on the picture of the formation.

6) The sculpture of spores

The spore-forms of sculpture-element, that is the reticulate, foveolate, verrucate-echinate ones, outlined by KRUTZSCH (1963) as a characteristic of recent Lycopodiaceae-spores, are frequent in case of the spores in the Lower Cretaceous.

On the basis of above literary data we have come to the conclusion that—supposing the botanical connection — we should treat of the spores of reticulate, foveolate, hamulate structures, found in our material, like of the fossil representatives of Lycopodiaceae. The recent spores of verrucate-echinate type are less frequent and less known; the Selaginellaceae-spores are of similar structure, as well. We do not deal, therefore, with the fossil spores of a structure, like this, in our present paper.

## Materials and Methods

The procedure of samples took place with Zólyomi—Kedves's method. The preparations were preserved in glycerin-jelly.
Determinations and countings are made with a Zeiss NfpK microscope.
Holotypes and figured specimens are lodged in the palynology collection of the Department of Botany, Attila József University, Szeged.

Systematic description

| | |
|---|---|
| Phylum | **PTERIDOPHYTA** |
| Class | **Lycopsida** |
| Order | **Lycopodiales** |
| Family | *Lycopodiaceae* |

Genus *RETITRILETES* (PIERCE, 1961) DÖRING, KRUTZSCH, MAI et SCHULZ, 1963 (short: D. K. M. S. 1963)

Syn.: *Lycopodiumsporites* THIERG. ex DELCOURT et SPRUMONT 1955.

Type species: *Retitriletes globosus* PIERCE, 1961.

Remarks: Some authors have included in the formgenera *Retitriletes* and *Lycopodiumsporites* the spores of Lycopodiaceae-affinity whose distal surface is reticulate and proximal surface is mostly smooth (non-reticulate). In the English palynologic literature the name *Lycopodiumsporites* is used almost exclusively, not accepting the opinion of DÖRING et al. (in KRUTZSCH, 1963, p. 11.) summarized as follows:

1) KRUTZSCH proved by re-examining Thiergardt's original slide (1963, Table 40, figs. 2—4) that the Niederlausitz-form of *Lycopodiumsporites agathoecus* (R. POT.) THIERG. 1938, considered as the type species of genus, is a recent *Lycopodium clavatum* L.

2) The Eocene form *Sporites agathoecus* (from Geiseltal), described by R. POTONIE in 1934, is not reticulate but of macrofoveate sculpture, and a form of Shizaeaceae affinity, being close to Foveasporis.

3) *R. Potonie,* in his SYNOPSIS (1956), gives "agathoecus" as the type-species of *Lycopodiumsporites* but his diagnosis and schematized diagram are approaching Thiergardt's form, corresponding to a recent species.

In that way, the genus hasn't type-species, whoever may be considered as an author.

Concerning *Retitriletes* it is to be noticed that PIERCE (1961) does not refer to the genus-name being taken over from van der HAMMEN.

By the emended diagnosis of DÖRING et al. (in KRUTZSCH, 1963), a form-genus, selected by them more or less arbitrarily and characterized but poorly, is made valid.

*Retitriletes tenuis* (BALME, 1957) n. comb.
Plate 1, Figs. 1, 2.

1957 *Lycopodiumsporites austroclavatidites tenuis* n. sp. BALME, p. 16., Pl. 1, Figs. 9—11.

1968 *Lycopodiumsporites tenuis* (BALME) n. comb. NORRIS, p. 319., Figs. 37—38.

Remarks: NORRIS (1968) was separating *Lycopodiumsporites tenuis* BALME from *Lycopodiumsporites austroclavatidites* as an independent form-species. The former species is generally of smaller size than 30 μ, the lumina of reticulum narrower, muri low, the trilete mark is less prominent.

Occurrence. Hungary: Gerecse Mts (Neocomian), Bakony Mts (Aptian—Lower Albian). Rare.

Plate 1. 1,2 *Retitriletes tenuis* (BALME, 1957) n. comb, Gerecse Mts, Borehole Süttő—3:125/3. P:32./102,9

3,4 *Retitriletes austroclavatides* (COOKSON, 1953) D. K. M. S. 1963, Bakony Mts, Tés, Borehole Té—27:46,0 /1.P:30,5./92,9

5,6 *Retitriletes clavatoides* (COUPER, 1958) D. K. M. S. 1963 Gerecse, Mts, Süttő—3:111/1 P:44,4) 100,3

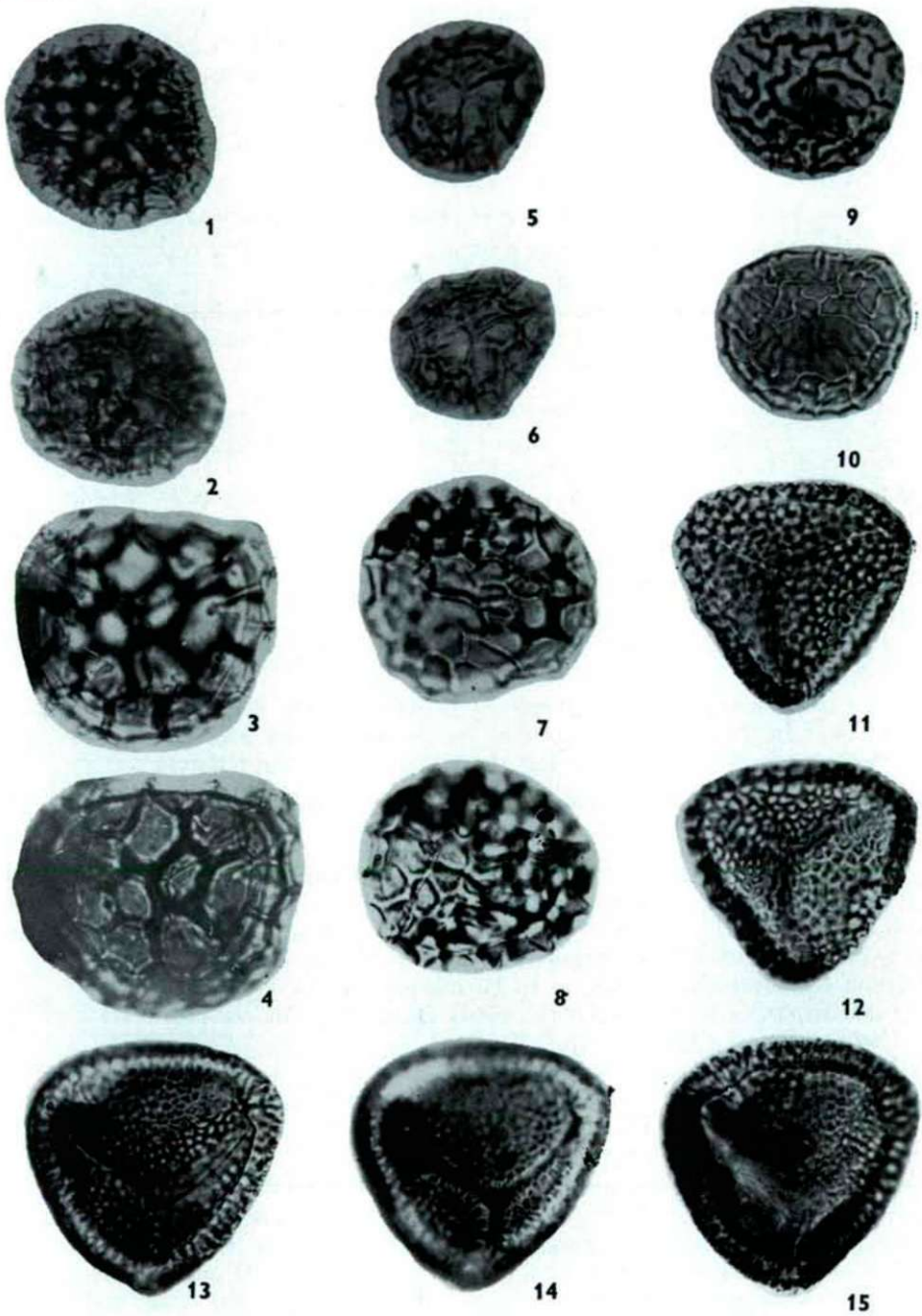
7,8 *Retitriletes dentimuratus* (BRENNER, 1963) n. comb, Bakony Mts, Hárskút, Borehole Hk—4:247 /1.P:40,2./107

9,10 *Retitriletes glebulentus* (KEMP, 1971) n. comb, Tatabánya Basin, Borehole Ta—1366:160 /1.P:36,2./97

11,12 *Vadaszisporites urkuticus* (DEÁK, 1964) DEÁK et COMBAZ 1967, Bakony Mts, Borehole Pe—27:82,7m /2.P:32./113,1

13,15 *Vadaszisporites urkuticus* (DEÁK, 1964) DEÁK et COMBAZ, 1967, Bakony Mts, Balinka. Borehole Ba—237:48 /4.P:46,6/ 112,1

Plate 1



*Retitriletes austroclavatidites* (COOKSON, 1953) D. K. M. S. 1963
Plate 1., Figs. 3, 4.

1953 *Lycopodium austroclavatidites* n. sp. COOKSON, p. 469, Pl.2, Fig. 35.
1963 *Retitriletes austroclavatidites* (COOKSON) n. c. DÖRING, KRUTZSCH, MAI, SCHULZ, p. 16.

Remarks: DETTMANN (1963) is giving a good description and synonim-list of this species.

Occurrence. Hungary: Gerecse Mts (Neocomian), Bakony and Vértes Mts (Aptian—Lower Albian)

*Retitriletes clavatoides* (COUPER, 1958) D. K. M. S. 1963
Plate 1., Figs. 5, 6.

1958 *Lycopodiumsporites clavatoides* n. sp. COUPER, p. 132, P 1.15., Figs. 10, 11.
1963 *Retitriletes clavatoides* (COUPER 1958) n. comb. DÖRING, KRUTZSCH, MAI et SCHULZ, p. 16.

Occurrence. Hungary: Gerecse Mts (Neocomian)

*Retitriletes dentimuratus* (BRENNER, 1963) n. comb.
Plate 1., Figs. 7, 8.

1963 *Lycopodiumsporites dentimuratus* n. sp. BRENNER, p. 44. P 1.5, Fig. 4.

Remarks: It is a form occurring comparatively rarely. The specimens in Hungary are generally somewhat smaller than the holotype.

Occurrence. USA: Maryland (Potomac group); Hungary: Bakony Mts (Upper Albian).

*Retitriletes glebulentus* (KEMP, 1971) n. comb.
Pl. 1., Figs. 9, 10.

1971 *Lycopodiumsporites glebulentus* n. sp. KEMP, p. 88—89, Pl. 12, Figs. 1—6.

Remarks: In the specimens examined by us, the circular area on the distal pole is anyway to be found but it is less thickened than in the holotype.

Occurrence. South England: Atherfield (Lower Albian); Hungary: Tatabánya Basin, Gerecse Mts (Lower Albian).

Genus *VADASZISPORITES* (DEÁK et COMBAZ, 1967) emend.

Emended diagnosis: azonotrilete miospores. Amb rounded to subtriangular, with convex or straight sides. Exosporium ornamented both proximally and distally with positive microreticulate-reticulate pattern. Lumina of reticulum somewhat polygonal in outline. The species to be included in the genus are:

1) *Vadaszisporites urkuticus* (DEÁK, 1964) DEÁK et COMBAZ, 1967
2) *Vadaszisporites gregussi* n. fsp.
3) *Vadaszisporites minutireticulatus* n. fsp.
4) *Vadaszisporites pseudofoveolatus* (DEÁK, 1964) DEÁK et COMBAZ, 1967
5) *Vadaszisporites sacali* DEÁK et COMBAZ, 1967
6) *Vadaszisporites* (al. *Microreticulatisporites*) *uniformis* (SINGH, 1964) n. comb.

Remarks: The genus created by DEÁK et COMBAZ (1967) is collecting the microreticulate-reticulate forms on both surfaces whose reticulum is positive, separated from the genus *Microreticulatisporites* (KNOX, 1950) R. POT. et KR. 1954, of "negative" reticulum (cf. KRUTZSCH, 1963, p. 11, 14, Fig. 9), and from *Retitriletes* (al. *Lycopodiumsporites*) having a positive reticulum but on the distal surface.

The authors of *Vadaszisporites,* in their genusdiagnosis, wrote about a "reticulate cingulum". But after examining a number of specimens it was proved that at these forms there is no cingulum only the wall of exosporium may be very thick.

The wall-thickness varies at the single species and even the different specimens of the same species may differ from one another in wall-thickness.

DEÁK (1964) was describing *Vadaszisporites urkuticus* and. *V. pseudofoveolatus* from the "Munieria marl" sediments in Bakony Mts, GÓCZÁN (in NAGY, 1971) and SCHOLZ (1974) placing these layers in the Albian.

*Vadaszisporites urkuticus* (DEÁK, 1964) DEÁK et COMBAZ, 1967.
Pl. 1., Figs. 11—15.

1964 *Microreticulatisporites urkuticus* n. sp. DEÁK, p. 106, Pl. 2, 14—15.
1967 *Vadaszisporites urkuticus* (DEÁK, 1964) n. comb. DEÁK et COMBAZ, p. 79, Pl. 1, Fig. 13.

Remarks: According our investigations, it appeared first at the upper level of dark-grey aleurolit of the Gerecse Mts and Tatabánya Basin (Lower Albian). GÓCZÁN (1964) found it at Upper-Maestricht substage, as well, and evaluated it there as a stratigraphically important formation.

The wall-thickness is, within the species, a very varying feature. In case of Upper Albian specimens the percentage of those having thicker exosporium is higher.

Occurrence. Hungary: Gerecse Mts, Tatabánya Basin (Lower Albian), Bakony, Vértes Mts (Upper Albian). In France: Saintogne (Upper Albian—L. Cenomanian).

*Vadaszisporites sacali* DEÁK et COMBAZ, 1967
Pl. 2., Figs. 1, 2.

1967 *Vadaszisporites sacali* n. sp. DEÁK et COMBAZ, p. 79, Fig. 14.

Remarks: This form is one of the characteristic formspecies of Upper Albian and Lower Cenomanian of Bakony Mts.

Exosporium of spore is usually profusely reticulate, with a thick wall. The lumina of microreticulum are narrow, pentagonal-or hexagonal.

Occurrence. Hungary: Bakony Mts (Upper Albian—Lower Cenomanian). France: Saintogne, Landun (Upper Albian—Cenomanian).

*Vadaszisporites gregussi* n. fsp.
Pl. 2., Figs. 3, 4.

Derivatio nominis: The specific epithet is given after Prof. P. Greguss, A. József University, Szeged.

Holotypus: Slide Pe—31: 351/2. P: 30,0/98,0 Pl. 2, 3, 4.

Locus typicus: Borehole Pe—31, Olaszfalu (Bakony Mts).

Stratum typicum: Turrilites marl (Upper Albian)

Diagnosis: Trilete miospore; amb subcircular to subtriangular, with convex sides, and rounded apices. Laesura straight, simple, lenght about 4/5 spore radius. Exosporium 3—4 μ thick. Both proximal and distal surfaces ornamented with regular, polygonal, positive reticulum. Lumina of reticulum 4—5 μ across, muri 0,5—1 μ wide and 1—1,5 μ wide at their bases; height 1—1,2 μ.

Size range: 48—54 μ.

Comparison: This species differs from the other Vadaszisporites species by its convex sides, larger lumina of reticulum and thicker muri.

Occurrence. Hungary: Bakony Mts (Upper Albian).

*Vadaszisporites uniformis* (SINGH, 1964) n. comb.
Pl. 2., Figs. 5, 6.

1964 *Microreticulatisporites uniformis* n. sp. Singh, Pl. 103, Figs. 15, 16.
1971 *Microreticulatisporites uniformis* SINGH 1964 PLAYFORD, p. 539, Pl. 103, Figs. 15, 16.

Remarks: We have thought good to transfer the form of positive reticulum described by SINGH (1964) to the genus *Vadaszisporites*, on the basis of the figure published and the present form being similar.

Occurrence. Hungary: Bakony Mts (Upper Albian); Canada Alberta (Albian).

*Vadaszisporites minutireticulatus* n. fsp.
Pl. 2., Figs. 7, 8.

Derivatio nominis: after its very fine microreticulate sculpture.

Holotypus: Slide Pe—27:82, 6—86,7 m /3. P: 35.7/ 108.7

Locus typicus: Borehole Pe—27. Olaszfalú, Bakony Mts
Stratum typicum: Turrilites marl (Upper Albian).

Diagnosis: Trilete miospore; amb subcircular to convexly subtriangular, with rounded angles.

Laesure straight, short, extending to 1/2 of spore radius. On the proximal and distal surfaces a very fine, positive microreticulate sculpture is to be observed. Lumina of microreticulum 0,3—0,5 μ across. Muri alway thinner than lumen in diameter. Exosporium 1—1,5 μ thick.

Size: 40—48 μ.

Comparison: This species differs from *Vadaszisporites uniformis* and *Vadaszisporites pseudofoveolatus* in its larger size, mostly rounded amb, and very fine microreticulate sculpture.

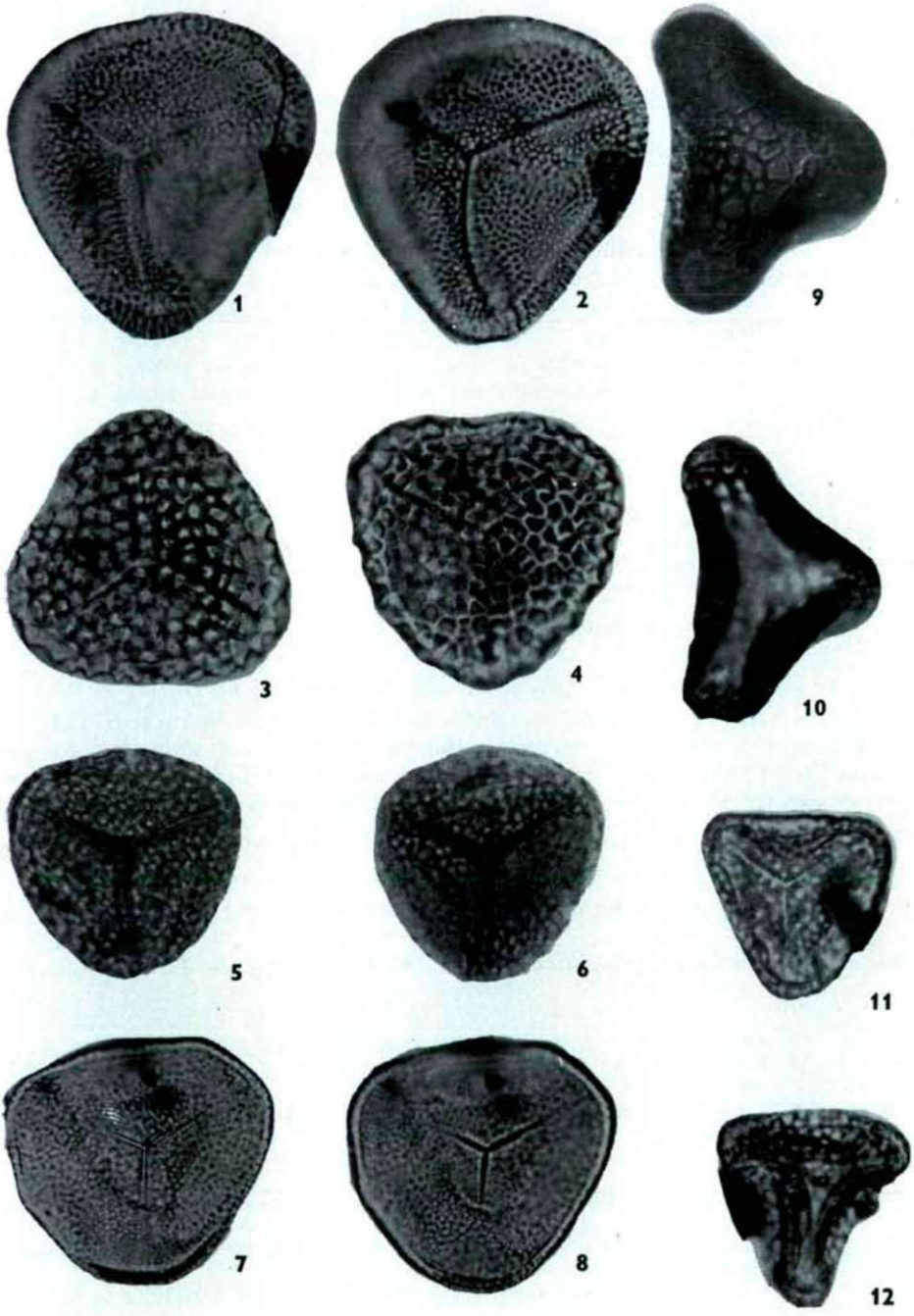
Occurrence. Hungary: Bakony Mts (Upper Albian—Lower Cenomanian). Occasional.

*Vadaszisporites* fsp.
Pl. 2., Figs. 9, 10.

Occurrence. Rare form from Turrilites marl of Bakony.

Plate 2. 1,2 *Vadaszisporites sacali* DEÁK et COMBAZ, 1967 Bakony Mts, Olaszfalu. Pe—31: 161/1.P:30,5./105,3
3,4 *Vadaszisporites gregussi* n. fsp. Bakony Mts, Olaszfalu. Pe—31:351 /2.P:30,0./98,0
5,6 *Vadaszisporites uniformis* (SINGH, 1964) n. comb. Bakony Mts, Olaszfalu. Pe—31: 333/2.P:39,5./108,7
7,8 *Vadaszisporites minutireticulatus* n.fsp. Bakony Mts, Olaszfalu. Pe—27:82,7 m/3. P:35,7./108,7
9,10 *Vadaszisporites* sp. Bakony Mts, Hárskút. Hk—4:10 /3.P:35,2./96,7
11,12 *Vadaszisporites pseudofoveolatus* (DEÁK, 1964) DEÁK et COMBAZ, 1967 Bakony Mts, Csehbánya. Cs—5:265 m /1.P:45,5./93,6

Plate 2



*Vadaszisporites pseudofoveolatus* (DEÁK, 1964) DEÁK et COMBAZ, 1967
Pl. 2., Figs. 11, 12.

1964 *Microreticulatisporites pseudofoveolatus* n. sp. DEÁK, p. 106—107, Pl. 2, Figs 16—18.

1967 *Vadaszisporites pseudofoveolatus* (DEÁK, 1964) n. comb. DEÁK et COMBAZ, p. 79.

Remarks: *V. pseudofoveolatus* is of the smallest size among the *Vadaszisporites* species. Most specimens have a kyrtom-like labra (Fig. 12), although the exosporium is thick enough (2—2,5 μ). It manifests itself already in the Lower Albian deposits.

Genus *FOVEOSPORITES* BALME 1957

Type species: *Foveosporites canalis* BALME 1957

Remarks: This genus was constructed by BALME (1957) for rounded to rounded-triangular spores of foveolate sculpture. *Foveotriletes* van der HAMMEN and *Microreticulatisporites* (KNOX) R. POT. et KR. distinct from *Foveosporites* BALME. The first genus having a concavely triangular amb, the latter genus having a reticulate sculpture.

*Foveosporites canalis* BALME 1957
Pl. 3., Figs. 1, 2.

1957 *Foveosporites canalis* n. sp. BALME, Pl. I., Figs. 15—17.

Occurrence. It is a frequent form in the Aptian to Albian deposits of the Tatabánya Basin and Gerecse Mts from among the Lower Cretaceous deposits in Hungary. It occurs but rarely together with the Vadaszisporites species.

*Foveosporites subtriangularis* (BRENNER, 1963) SCHULZ, 1966
Pl. 3., Figs. 3, 4.

1963 *Foveotriletes subtriangularis* n. sp. BRENNER, p. 62., Pl. 16, Fig. 2.

1966 *Foveosporites subtriangularis* (BRENNER 1963) n. comb. SCHULZ, p. 134.

Remarks: The foveae of form published by us are of somewhat narrower lumina than those of holotype. The arrangement of this form among the *Foveosporites* was carried out by KEMP (1971) and PHILLIPS et FELIX (1971), as well.

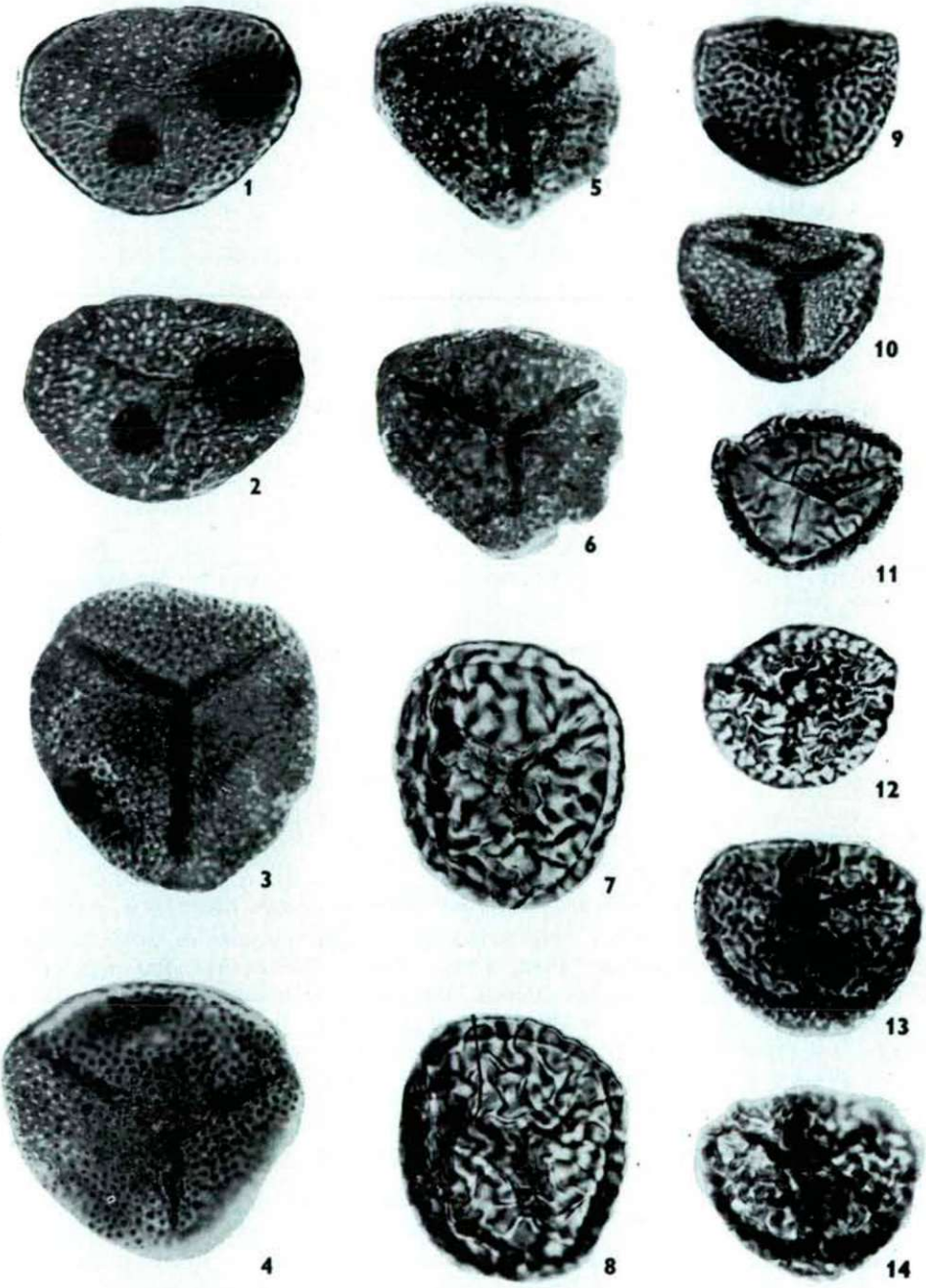
Occurrence. Hungary: Tatabánya Basin (Lower Albian). USA: Maryland (Potomac Group). England: Redcliff (Upper Aptian).

*Foveosporites multifoveolatus* DÖRING, 1965
Pl. 3., Figs. 5, 6.

1965 *Foveosporites multifoveolatus* n. fsp. DÖRING, p. 43—44, Pl. 15, Figs. 3—5.

Plate 3. 1,2 *Foveosporites canalis* BALME1957 Gerecse Mts, Süttő—3:13 /3.P:38,9./101,8
3,4 *Foveosporites subtriangularis* (BRENNER, 1963) SCHULZ, 1966 Tatabánya Basin. Ta—1358:369,5 m /2. P:44,6./101,5
5,6 *Foveosporites multifoveolatus* DÖRING, 1965 Tatabánya Basin. Ta—1329:461,5 m/2. P:42,2./111,1
7,8 *Camarozonosporites insignis* NORRIS, 1967 Bakony Mts, Csehbánya. Cs—5:273 m/1. P:30,5./108,9
9,10 *Sestrosporites pseudoalveolatus* (COUPER, 1958) DETTMANN, 1963 Tatabánya Basin. Környe. Kö—24:245 /1. P:42,0./101,0
11,12 *Camarozonosporites cerniidites* (ROSS, 1949) W. KR. 1959 Bakony Mts. Hárskút. Hk—4:641 /2. P:38,7./111,9
13,14 *Camarozonosporites* sp. Gerecse Mts, Süttő—3:119 /1. P:43,4./107,1

Plate 3



Occurrence. Döring (1965) described it from Westmecklenburg, from the uppermost stage of the layers Wealden. In Hungary, it is frequent in the Neocomian deposits of Gerecse Mts and occurs in the Aptian, as well. RÁKOSI (1971) described it from the Neocomian deposits of Dorog Basin.

## Genus *SESTROSPORITES* DETTMANN 1963

Type species: *Sestrosporites irregulatus* (COUPER, 1958) DETTMANN, 1963.

This genus was applied by DETTMANN (1963) to the proximally smooth, distally foveolate-foveoreticulate trilete spore-forms whose exosporium in the interradial regions became thicker.

### *Sestrosporites pseudoalveolatus* (COUPER, 1958) DETTMANN, 1963

Pl. 3., Figs. 9, 10.

1958 *Cingulatisporites pseudoalveolatus* n. sp. Couper, p. 147, Pl. 25., Figs. 5, 6.
1963 *Sestrosporites pseudoalveolatus* (COUPER) n. comb. DETTMANN, p. 66, Pl. 13, Figs. 11—16.

Remarks: This form was found by COUPER (1958), GUY (1971) in the Jurassic deposits, by DETTMANN (1963), SINGH (1964), NORRIS (1967), KEMP (1971), ČORNA (1970, 1972) in the Aptian and Albian deposits.

In Hungary it was found in the Lower Albian deposits of Tatabánya and Gerecse Mts.

## *Genus CAMAROZONOSPORITES* (PANT ex R. POT., 1956) KLAUS, 1960

Type species: *Camarozonosporites cretaceus* (WEYLAND et KRIEGER, 1953) R. POT. 1956.

### *Camarozonosporites insignis* NORRIS, 1967

Pl. 3., Figs. 7, 8.

1967 *Camarozonosporites insignis* n. sp. NORRIS, p. 96, Pl. 13, Figs. 14—16.

Occurrence. Hungary: Bakony Mts (Middle Albian). USA: Maryland (Albian—Cenomanian). Canada: Alberta (Albian—Cenomanian). England: Warlingham (Aptian—Albian).

### *Camarozonosporites cerniidites* (ROSS, 1949) W. KR. 1959

Pl. 3., Figs. 11, 12.

1949 *Lycopodium cerniidites* n. spm. ROSS, p. 30—31, Pl. 1, Figs. 1—2.
1959b *Camarozonosporites cerniidites* (ROSS, 1949) n. comb. KRUTZSCH, p. 187.

Remarks: *C. cerniidites* were placed by various authors in different form-genera. COUPER (1958) ranged them among *Lycopodiumsporites*, BRENNER (1963) among *Lycopodiacidites*. In our opinion, owing to the hamulate sculpture of the distal surface of the form, it is absolutely justified if we put in *Camarozonosporites* genus. It is placed by KRUTZSCH (1963, p. 22) even at subgenus level in the *Camarozonosporites* (*Camarozonosporitess*) subgenus that contains only those having hamulate forms on their distal surface.

Occurrence. Hungary: Bakony Mts (Upper Albian—Cenomanian).

### *Camarozonosporites* sp.

Pl. 3., Figs. 13, 14.

Remarks: The form published is somewhat similar to the form *Lycopodiacidites arcuatus* HEDLUND 1966 its distal surface is, however, of hamulate sculpture

where the sculpture-elements are showing, in some places, a "corrugate" form. The proximal surface is smooth, divided into three areas. The sides of spore became thick interradially.

Occurrence. Hungary: Gerecse Mts (Lower Albian)

## Conclusion

In the sporomorpha-associations of the Lower Cretaceous deposits in Hungary, the spores of Lycopodiaceae-affinity, emphasized by us and published above, don't play any significant part. It may be supposed that in that Period Lycopodiaceae family, even if it had the due species number, did not form any association.

In the Neocomian and Aptian deposits *Retitriletes austroclavatidites, Retitriletes clavatoides, Foveosporites canalis, Foveosporites multifoveolatus* can be found. As these forms are distributed enough in time and space (some species being present in the Jurassic deposits) as well, and in the Lower Cretaceous age having a wide geographical distribution, it is to be supposed that the mother-plants of these spores accomodated themselves well to the ecologic and climatic conditions, were species of cosmopolitan character.

The great majority of the formspecies *Vadaszisporites* appeared in the Middle and Upper Albian. The ecologic claim of these Lycopodiaceae species may already have been connected with the warm, more humid, subtropical-tropical climate, possibly with the gallery-forestlike open plant associations.

## References


BALME, B. E. (1957): Spores and pollen grains from the Mesozoic of Western Australia. — C.S.I.R.O., Coal Res. Sect. *25*, 48.

BRENNER, G. J. (1963): The spores and pollen of Potomac Group of Maryland. — Bull. Md. Dep. Geol. Mines *27*, pp. 215.

COUPER, R. A. (1958): British Mesozoic microspores and pollen grains, a systematic and stratigraphic study. — Paleontographica B., *103*, 75—179.

DEÁK, H. M. (1964): Contribution à l'étude palynologique du groupe d' argiles à Munieria de l'étage Aptien. — Acta Bot. Hung. *10*, 95—126.

DEÁK, H. M., et COMBAZ A. (1967): "Microfossiles organiques du Wealdien et du Cénomanien dans un sondage de Charente—Maritime. — Rev. Micropal. *10*, 69—96.

DETTMANN, M. E. (1963): Upper Mesozoic microfloras from southeastern Australia. — Proc. Roy. Soc. Vict. *77*, 1—148.

DÖRING, H. (1965): Die sporenpaläontologische Gliederung des Wealden in Westmecklenburg (Struktur Werle). — Geologie *14*, 1—118.

ENGLER, A., und PRANTL, K. (1902): Die natürlichen Pflanzenfamilien. (PRITZEL, E.: III. Lycopodiales, p. 563—606.). — Leipzig, Verlag W. Engelmann.

FÜLÖP, J. (1958): Die kretazeischen Bildungen des Gerecse Gebirges. — Geol. Hung., s. Geol. *11*, 1—124.

FÜLÖP, J. (1964): Unterkreide — Bildungen (Berrias—Apt) des Bakony-Gebirges. — Geol. Hung., s. Geol., *13*, 1—194.

FÜLÖP, J. (1966): Les formations Crétacées de la Montagne de Villány. — Geol. Hung., s. Geol. *15*, 1—131.

GÓCZÁN, F. (1964): Stratigraphic palynology of the Hungarian Upper Cretaceous. — Acta Geol., Budapest, 229—264.

HARRIS, W. F. (1955): A manual of the spores of New Zealand Pteridophyta. — Bull. N. Z. Dep. Sc. ind. Res. *116*, 1—186.
HERTER, W. (1909): Beiträge zur Kenntnis der Gattung *Lycopodium*. — Engl. Bot. Jb. *43*.
KEMP, E. M. (1971): Aptian and Albian microspores from southern England. — Paleontographica B. *131*, 73—143.
KNOX, E. (1950): The spores of *Lycopodium, Phylloglossum, Selaginella* and Isoätes and their Value in the Study of Microfossils of Paleozoic age. — Transact. and Proc. Bot. Soc. *35*, 207—357.
KRUTZSCH, W. (1959): Mikropaläontologische (sporen-paläontologische) Untersuchungen in der Braunkohle des Geiseltales. — Geologie, Beih. *22*, 1—425.
KRUTZSCH, W. (1963): Atlas der mittel- und jungtertiären dispersen Sporen- und Pollen sowie der Mikroplanktonformen des nördlichen Mitteleuropas. — Berlin.
NAGY, E. (1971): The Paleontological Section's work in 1968. — Rel. Ann. Inst. Geol. Publ. Hung. p. 344—347.
NESSEL, H. (1939): Die Bärlapp gewäsche (Lycopodiaceae). — Jena.
NORRIS G. (1967): Spores and pollen from the lower Colorado Group (Albian—Cenomanian) of central Alberta-Paleontographica B. *120*, 72—115.
NORRIS, G. (1968): Plant microfossils from the Hawk Crag Breccia, south-west Nelson, New Zealand. — N. Zeal. J. Geol. Geophys. *11*, 312—344.
PIERCE, R. L. (1961): Lower Cretaceous plant microfossils from Minnesota. — Bull. Minn. Geol. Surv. *42*, 86.
PLAYFORD, G. (1971): Palynology of basal Cretaceous (Swan River) strata of Saskatchewan and Manitoba. — Paleontology *14*, 533—565.
RÁKOSI, L. (1971): Palynologische Untersuchung des Neokomuntergrundes des Doroges Braunkohlen. — Rel. Ann. Inst. Geol. Publ. Hung. 267—292.
SCHOLZ, G. (1974): Beitrag zur Kenntnis der Mittelkreide Bildungen im nördlichen Bakony-Gebirge. — Bull. Hung. Geol. Soc. *104*, *344—347*.
SCHULZ, E. (1965): Über einige neue Sporae dispersae aus dem älteren Mesophytikum Deutschlands. — Geologie, Beih. *55*, 130—151.
SINGH, C. (1964): Microflora of the Lower Cretaceous Mannville Groups, East-Central Alberta. — Bull. Res. Coun. Alberta *15*, 238.

Address of the author:
Dr. M. JUHÁSZ
Department of Botany, A. J. Univetsity
H—6701 Szeged, P. O. Box 428,
Hungary

Acta Biologica Szeged. *21* (1—4), pp. 35—40 (1975)

# INFLUENCE OF HISTOLOGICAL FACTORS ON STALK STRENGTH IN MAIZE

L. KÁLMÁN, J. NÉMETH, Á. KOROM, and I. MARÓTI

*Cereal Research Institute, Szeged* and *Department of Botany, Attila József University, Szeged*

(Received September 12, 1974)

**Abstract**

For 10 maize genotypes, the percentage of broken stalks two weeks after harvesting, and crushing strength of 5 cm sections of the second internode were determined and crushing strength was compared with histological structure.

The primary determinant of mechanical stalk strength appeared to be, beyond the rind thickness, the thickness of the sheath of the vascular bundles at the periphery of the stalk. The number of vascular bundles did not differ, except in the peripheral zone, where more were present in weak-stalked types.

In the last few years the increased stalk-breaking in maize, that caused considerable yield loss in Hungary was considered to be result of monoculture, mechanized harvesting, application of large amounts of nitrogen fertilizer (above 160 kg/ha), and higher plant population.

Research workers searching for the causes of stalk-breaking usually discuss the problem from the point of view of their own special field of research, emphasizing the overhelming importance of one of the factors possible that may be important.

The fungi reported by SZÉCSI (1973), *Fusarium roseum* var. *graminearum* and *Fusarium roseum* var. *culmorum* were the cause of root-and stalk-rot in corn in Hungary.

KOEHLER et al. (1925), FOLEY (1960), and WILCOXSON (1962) observed that many broken stalks were not badly rotted, yet rotted stalks still stood upright. NELSON (1958) suggested distinguishing between two types of stalk-breaking, i.e., caused by susceptibility to fungi on the one hand, and caused by the poor mechancal strength of stalk tissue on the other.

In order to estimate stalk strength, and often stalk-rot too, for many years only a single method was used, namely a percentage of broken stalks — broken above the ear — of a given genotype. This method provides a measure of the gross character, but provides little information concerning the relative importance of component factors, and it is seriously dependent upon environmental factors.

ZUBER and GROGAN (1961) introduced a mechanical method applicable for quantitative measuring. This crushing-strength measurement provides reliable information in regard to stalk strength of individual plants, because crushing-strength is significantly correlated with standing upright and rind thickness and weight of 5 cm sections of second and third internodes.

The question is raised whether existence of various types showing a wide range of stalk quality may be explained on the basis of the fine structure of stalk.

Inbred lines differing in lodging behaviour were studied by HUNTER and DALBEY (1937) in the field and in the laboratory. Correlations between anatomical structure and field behaviour were found. Strong-stalked lines possessed thick layers of deeply stained sclerenchyma, both around the vascular elements and in the subepidermis, and had angular cells with small intercellular spaces.

McROSTIE and MACLACHLAN (1942) noted that strong-stalked corn had much lignification beyond the rind and a large number of vascular bundles within the lignified area. MAGEE (1948) reported that strong-stalked types had a low bundle number per square millimeter in the rind, a high percentage of sheath per bundle, a large stalk diameter, and a wide lignified zone. FOCKE and KUHFUSS (1961) concluded that resistance to lodging appeared to be associated with an increase in stalk lignification both in the peripheral tissues and in the vascular bundles.

BOOTHROYD (1962) concluded that stalk strength was not associated with the histological aspects considered which include the percentage of sclerenchyma sheath fibres per vascular bundle and per unit area of the rind. NELSON (1958) established that genetical improvement of stalk strength had not been accompanied by the change in number of vascular bundles, neither in the rind nor in the pith. According to CHANG and LOESCH (1972) the genotypic correlation between field lodging and bundle number in pith was essentially nil.

In the present work we studied the relationship between mechanical stalk strength and morphological components of the stalk.

## Materials and Methods

Five strong-stalked single crosses (A223×B14, EPI×W79A, Bc5[1]×GK4[2], A90×153R Fv2×153R) and five weak-stalked single crosses (A619×A632, A223×Kb6[3], Kb6×K71[4], Exp.29 F7×GK1) were chosen on the basis of previously observed stalk lodging responses. The hybrids were planted in a simple randomized block design with four replications. The spacing in the case of hybrids belonging to the early—maturity hybrids were planted in rows 70 cm apart and 25 cm between plants within rows whereas the later—maturing hybrids were spaced 70 cm between and 30 cm within rows.

*1)* Line released to cooperators by Zagreb.
*2)* Lines used by the Cereal Research Institute of Szeged
*3)* Lines used by the Institute for Irrigation in Szarvas, Hungary
*4)* Lines used formerly by the University of Agricultural Sciences of Keszthely, Hungary.

The percentage of stalk-breakage was recorded two weeks post harvesting (which occurred when grain moisture was 28 per cent). Stalks samples were taken from the second internode above soil level and dried at 40 °C for seven days. For the determination of mechanical stalk strength, five cm sections were cut from each internode.

Crushing strength was measured with an oil hydraulic press, while rind thickness was evaluated with a micrometer according to the method introduced by ZUBER and GROGAN (1961).

Six stalk sections of each hybrid were used for the histological study and the six observations were averaged. The stalks one centimeter thick were sawed from the center of each internode. Sections were boiled in distilled water for 8—10 hours and sectioned with a freezing microtome. After a histochemical staining (malachit green, toluidin blue) the sections were covered in gelatin paraffin. Besides micro—photographs some sections were photographed by projecting their image onto film with an enlarger.

## Results and Discussion

Our measurements showed that the types resistant to stalk-breaking belonged to the interval 131—190 kilopounds, and the weak-stalked types belonged to the interval 50—115 kilopounds (Table 1).

*Table 1.* Percentage of stalk breakage and crushing strength and rind thickness of the second internode of the strong- and weak-stalked genotypes

| Genotypes | Percentage of stalk breakage two weeks after harvesting ($H_2O$ % at harvest = 28 | Crushing strength (kp) | Rind thickness (mm) |
|---|---|---|---|
| A223×B14 | 1.3 | 151 | 1.634 |
| EP1×W79A | 5.3 | 190 | 1.235 |
| Bc5×GK4 | 5.7 | 131 | 1,267 |
| A90×153R | 6.2 | 175 | 1.428 |
| Fv2×153R | 6.6 | 150 | 1.065 |
| A619×A632 | 25.0 | 94 | 1.392 |
| A223×Kb6 | 57.1 | 50 | 1.079 |
| Kb6×K71 | 64.0 | 115 | 1.116 |
| Exp.29* | 65.8 | 54 | 1.097 |
| F7×GK1 | 84.0 | 79 | 0.925 |
| SzD% | | 41 | 0.151 |

* closed pedigree

According to data obtained out of the literature and our earlier results, there is a significant correlation between mechanical strength and rind thickness (ZUBER and GROGAN, 1961).

The "rind" represents a peripheral part of the stalk section which may be separated from the pith even mechanically after drying. It is a darker cylindrical ring.

The area of rind of the second internodes was estimated at 17—24 p. c. The area of rind in the case of strong-stalked genotypes was somewhat larger than in the case of weak-stalked ones. But the mechanical strength of the stalk can not be explained merely by thickness of the rind.

As a result of our investigations we concluded that the fine structure of the rind has an important role in making the stalk strong. Probably stalk strength is dependent on the proportion of lignified tissue elements.

If a stalk section is magnified a few times, it is clear that the collaterally closed vascular bundles are closer together near the periphery than in the central parenchymatic ground tissue which consits of larger cells with relatively thin walls. Distribution of the vascular bundles was counted in 0,5 mm broad zones from the epidermis to pith, and density was recorded in a columnal diagram (Fig. 1). The number

of vascular bundles in the first zone was higher in the case of the weak-stalked genotypes than in the strong-stalked ones. The density of vascular bundles in the second, third, and fourth zones was nearly the same.

Being merely 40 – 60 μ thick, even in the lower internodes, the hypodermal parenchyma is presumably of little importance in strengthening the stalk. The sclerified parenchyma is more important among the strengthening tissues. The rind consists mostly of this tissue and it may be regarded as a transitional zone between ground parenchyma and hypodermal sclerenchyma. The sclerified parenchyma was, on an average, 0,2 – 0,3 mm larger in the strong-stalked genotypes than in the weak-stalked ones. The peripheral zone consisting of smaller cells and intercellular spaces is important not only in strengthening the stalk but also in the transport of metabolites as well.

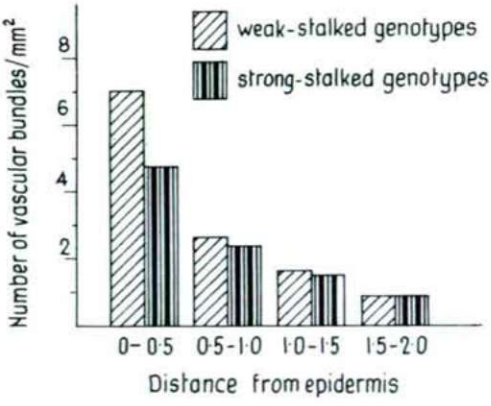



Fig. 1. Distribution of vascular bundles in different zones between rind and pith in the weak- and strong-stalked genotype

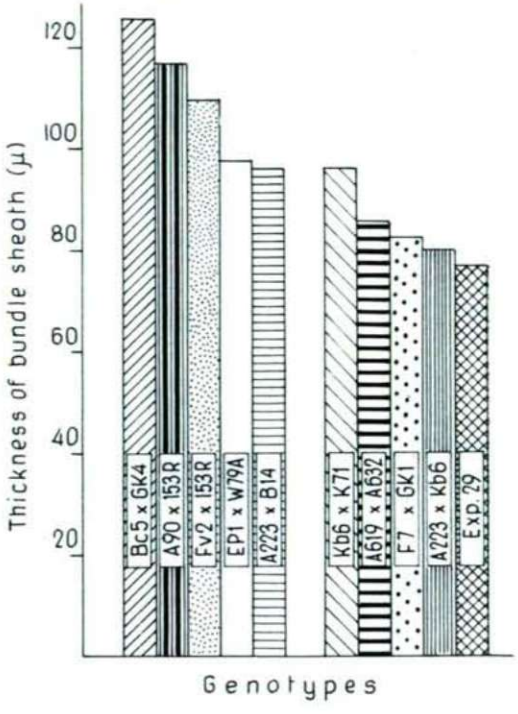



Fig. 2. The avarage thickness of bundle sheaths in the 2 mm zone from epidermis of weak- and strong-stalked genotypes

During the ripening period, when most of the cells in the pith die off because of Fusarium infection, the water transport in the plant is hardly disturbed, as the 50—60 p. c. of vascular bundles located in the rind (Table 2) continue to function.

*Table 2.* Percentage of the rind area of the cross section of 2. internode and the percentage of the vascular bundles in the rind and pith of the 2. internode at the different hybrids

| Combinations | Percentage of the rind area of the cross section of 2. internode | Percentage of the vascular bundles in the rind and pith of the 2. internode |
|---|---|---|
| A223×B14 | 23.9 | 53—47 |
| EP1×W79A | 20.8 | 46—54 |
| Bc5×GK4 | 20.6 | 55—45 |
| A90×153R | 23.3 | 54—46 |
| Fv2×153R | 21.2 | 54—46 |
| A619×A632 | 23.1 | 60—40 |
| A223×Kb6 | 18.9 | 42—58 |
| Kb6×K71 | 18.9 | 54—46 |
| Exp.29 | 19.3 | 50—50 |
| F7×GK1 | 17.2 | 54—46 |

The third strengthening tissue is the bundle sheath. This sheath, consisting of fibres of procambial origin, can continue to thicken as a result of sclerification of parenchyma cells. The thickness of bundle sheaths was, on an avarage, 20—30 μ thicker in the strong-stalked genotypes than in the weak-stalked ones (Figs. 2, 3).

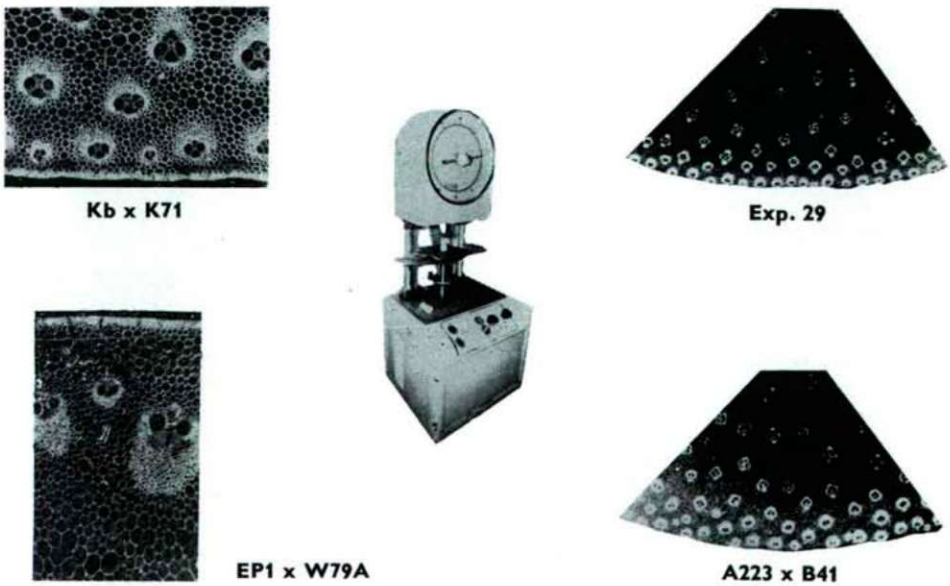



Fig. 3. Cross sections of stalk of strong — and weak — stalked hybrids and the hydraulic press.

## Literature

BOOTHROYD, C. W. (1962): Histological structure of corn stalks in relation to field resistance to stalk rot. Phytopathology *52*, 726 (Abstr.)

CHANG, HUI-SHONG, and LOESCH, P. J. JR. (1972): Genetic variation of four anatomical traits and their association with stalk-quality traits in maize (*Zea mays* L.). — Crop Science *12*, 271—274.

FOCKE, R. and KUHFUSS, K. H. (1961): Beobachtungen über Umbruchschaden bei Mais unter Einwirkung von Erdraupenfrass. — Nachr. Bl. Dtsch. Pfl. Sch. Dienst, Berlin *15*, 184—187.

FOLEY, D. C. (1960): The response of corn to inoculation with Diplodia zeae and Gibberella zeae. — Phytopathology *50*, 10 (Abstr.).

HUNTER, J. W. and DALBEY, N. E. (1937): A histological study of stalk-breaking in maize. — American Journal of Botany *24*, 492—494.

KOEHLER, B. G., DUNGAN, H. and HOLBERT, J. R. (1925): Factors infuencing lodging in corn. III. — Agr. Exp. Sta. Bull. 354.

MAGEE, J. A. (1948): Histological structure of the stem of *Zeae mays* in relation to stiffness of stalk. — Iowa State Coll. J. Sci. *9*, 489—503.

MC ROSTIE, G. P. and MACLACHLAN, J. D. (1942): Hybrid corn studies. — J. Sci. Agr. *22*, 307—313.

NELSON, C. E. (1958): Lodging of field corn as affected by cultivation, plant population, nitrogen fertilizer, and irrigation treatment. — U. S. Dept. Agr. Production Res. Rept. No. 16.

NELSON, S. W. (1968): Observed and expected response of yield and stalk strength to full-sib family selection in a population of maize. Ph. D. Thesis. — North Carolina State University (Libr. Congress Card Mic. 68—17. 567; Univ. Microfilms, Ann Arbor, Mich, Diss. Abstr. *29*, 4534-B).

SZÉCSI, Á. (1973): *Fusarium roseum* var. *graminearum* és *F. roseum* var. *culmorum* izolátumok celluláz aktivitása talajkultúrában. — Növénytermelés *22*, 103—106.

WILCOXSON, R. D. (1962): Stalk rot in relation to yield of corn. — Phytopathology *52*, 416—4188.

ZUBER, M. S. and GROGAN, C. O. (1961): A new technique for measur.ng stalk strength in corn. Crop Sci. 1:380.

Address of the authors:
Dr. L. KÁLMÁN
Dr. J. NÉMETH
Dr. Á. KOROM
Cereal Research Institute
H-6726 Szeged,
Dr. I. MARÓTI
Department of Botany, A. J. University
H-6701 Szeged, P. O. Box 428,
Hungary

Acta Biologica Szeged. *21* (1—4), pp. 41—42 (1975)

# NEW CHARACTERISTICS IN THE SUBMICROSCOPIC EXINE STRUCTURE OF THE POLLEN GRAINS OF NYMPHAEACEAE FROM AN EVOLUTIONARY POINT OF VIEW

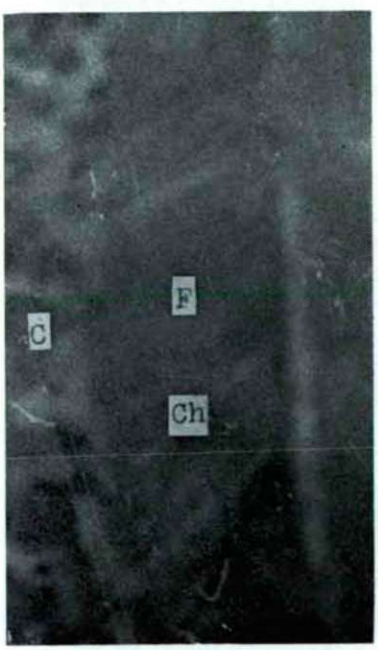
**(Short communication)**

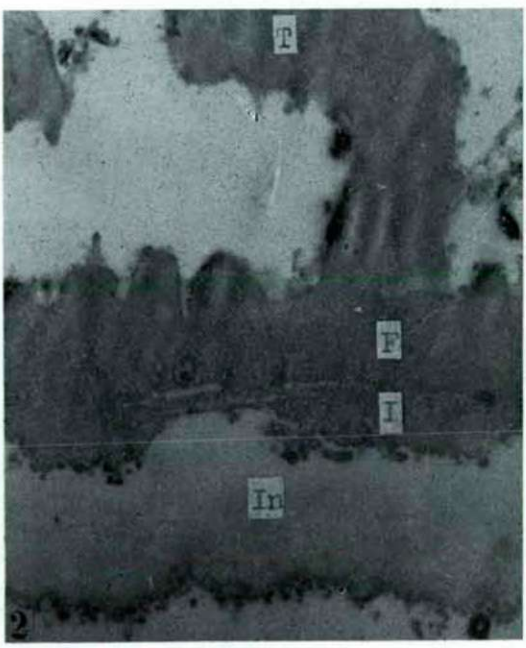
M. KEDVES and J. ANTUNOVICS

*Department of Botany, Attila József University, Szeged*

(Received August 27, 1974)

We have carried out light- and transmission-electronmicroscopic investigations on various Nymphaeaceae pollen grains. The transmission-electronmicroscopic investigations have led to two interesting results. In the foot layer of *Nuphar luteum* (L.) SM., we succeeded in observing very narrow channels, which is an unparalleled phenomenon in case of Dicotyledonopsida exines. It is a similar, but not identical characteristic that the foot layer perforated occurs in the monocotyledonous Restionaceae pollen grains (CHANDA and ROWLEY, 1967). In the exine of *Nelumbo lutea*




1. A detail of the ectexine of *Nuphar luteum* (L.) SM. In the foot layer, near the long spinae, there are narrow channels of askew course. M: ×100,000.
2. Ultrastructure of the exine of *Nelumbo lutea* PERS. M: ×25,000.
T-tectum, C-columellae, F-foot layer, I-interbedded zone, In-intine, Ch-channels.

PERS., we succeeded in demonstrating the interbedded zone, described by FREAN (1973) in pollen grains of the genus Croton.

The characteristics observed confirm to some extent the opinions concerning the evolutionary connection of Nymphaeaceae and, on the other hand, indicate that the pollen grains of Nymphaeceae are heterogenous with respect not only to their light-microscopic morphology but to the ultrastructure of the exine as well.

## References

CHANDA, S.—ROWLEY, J. (1967): Apertural types in pollen of the Restionaceae and Flagellariaceae. — Grana Palynologica *7*, 16—36.

FREAN, M. L. (1973): Exine stratification and fine structure of the pollen wall of *Croton gratissimus* (BURCH.) subsp. *subgratissimus* (PRAIN) BURTT DAVY. — Pollen et Spores *15*, 353—362.

Address of the authors:
Dr. M. KEDVES
J. ANTUNOVICS
Department of Botany, A. J. University, H-6701 Szeged, P. O. Box 428, Hungary

Acta Biologica Szeged. *21* (1—4), pp. 43—62 (1975)

# POLLEN GRAINS OF THE INTERPOROPOLLENITES FGEN. FROM SEDIMENTS OF THE UPPER CRETACEOUS PERIOD IN PORTUGAL

M. KEDVES and MÁRIA HEGEDŰS

*Department of Botany, Attila József University, Szeged*

(Received December 28, 1973)

**Abstract**

This paper deals with the taxonomy of the pollen grains of Interporopollenites fgen. from three habitats (Arada, Aveiro, Preza) in Portugal. Twenty-six form-species were present twenty-two of these new ones. The habitats investigated from a characteristic facies or level within the Upper Cretaceous Period, that is primarily characterized by being rich in these pollen grains.

## Introduction

The Interporopollenites genus was described by WEYLAND and KRIEGER (1953) from sediments of the Upper Cretaceous Period at Aachen. THOMSON and PFLUG (1953) classified all the primary short-axial angiospermous pollens, including the new species belonging to this genus and described by them, into the collective genus Extratriporopollenites PFLUG 1953a. PFLUG (1953b), in the course of founding the Normapolles stemma genera, applied the original name again. GÓCZÁN, GROOT, KRUTZSCH and PACLTOVÁ (1967), in re-elaborating the Normapolles genera, gave us a new description of that genus, too. The stratigraphic significance of these pollen grains arose particularly in KRUTZSCH's paper (1966) reporting on several fsp. names whose detailed description is still missing. KEDVES and DINIZ (1967), in their preliminary report on the sporomorphs of sediments from the Upper Cretaceous Period at Aveiro, established the next geological period (Santon/Campan), inter alia on the basis of the abundant occurrence of this form-genus. Several other publications have since pointed out the pollen grains of this genus from numerous habitats, mainly from sediments of the Upper Cretaceous, but also from the Lower Tertiary Period. The initial description however, have, not been followed by new ones. HEGEDŰS, KEDVES and PÁRDUTZ (1972) described a new species from the Aveiro habitat and investigated its exine ultrastructure too. The ultrastructure results threw new light upon the earlier, exclusively light-microscopic results in the case of this genus.

During the spore-pollen investigation of several habitats in Portugal from the Upper Cretaceous Period, the sporomorphous composition of "Aveiro type" was found in further habitats (Arada and Preza). Among the Angiospermatophyte pollen grains the large number and variety of forms of these are very remarkable. From the extremely rich material, in this paper we describe the pollen grains of the Inter-

poropollenites fgen.; this will be followed by other publications in this way eventually giving a complete picture about the sporomorphous composition of the habitats investigated.

## Materials and Methods

The pollen grains of the three habitats mentioned in the introduction, that are to be classified in the Interporopollenites fgen., are described in the following way. For the exine structure of course, we also use the experiences obtained during the electronmicroscopic investigations. As concerns the exine, we use the nomenclature that is already to be regarded as general in ultrastructure investigations. Unfortunately, many of them can be observed only with a light-microscope, but the caracteristic possibly occurring in their described forms is also doubtful; for instance, the spinae on the tectum, or the channel's in it. It is obvious, therefore, that the light-microscopic descriptions cannot be perfect in the present state of our knowledge. In the diagnoses, founded in this case exclusively on light-mosecroscopic observations, only the observed marks are given. These will presumably be completed in the near future with the TEM and SEM diagnoses, only on the basis of which we can get a complete picture of a pollen form-species. We think, however, that in the case of stratigraphic and palaeoecologic investigations the method applied in present work is also convenient, giving a good basis for comparison in the following investigations.

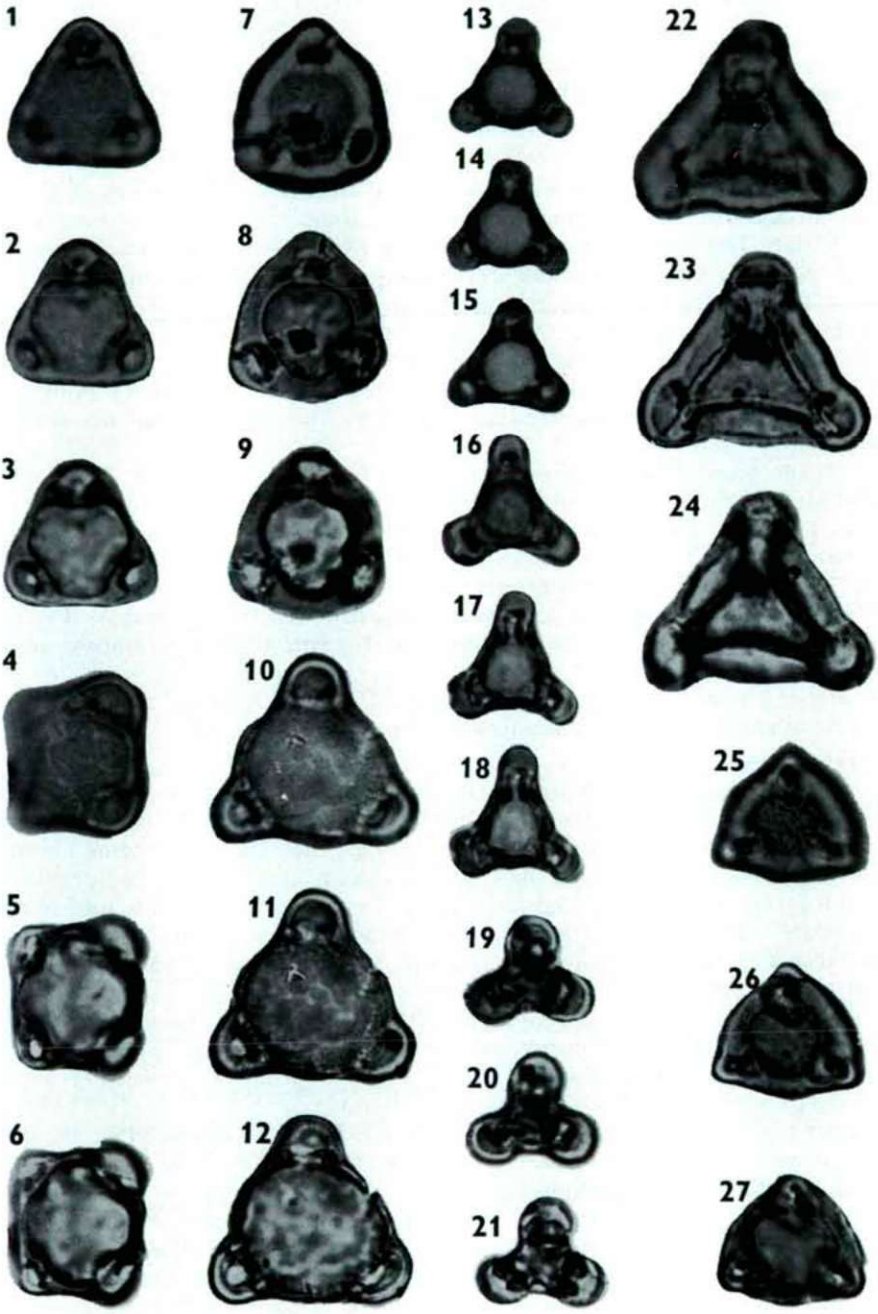
## Results

The pollen grains demonstrated can be classified into two large groups: homopolar pollen grains (with the same figurations on both surfaces) and heteropolar ones. The homopolar pollen grains can be divided into two further morphological groups: (1) both surfaces are without decoration, smooth; (2) both surfaces are decorated with the same figurations. The pollen grains demonstrated are described in this morphological order.

1.1 *Interporopollenites initium* (Pf. 1953a) Pf. 1953b (Plate 1, 1—6).

Plate 1. 1-3. — *Interporopollenites initium* (P . 1953a) P . 1953b, prep. Preza—III—3; cross-table No. 11.3-104.3.
4-6. — *IIIIInterporopollenites initium* (P . 1953a) P . 1953b, forma tetraexitus, prep. Preza—III—10; cross-table No. 8.8/115.8.
7-9. — *Interporopollenites endotriangulus* H , K and P 1972, prep. Aveiro—6; cross-table No. 13.2/115.0.
10-12. — *Interporopolienites subgranulosus* n. fsp., prep. Preza—III—18; cross-table No. 15.8/107.4.
13-15. — *Interporopollenites vancampoae* n. fsp., prep. Preza—III—6; cross-table No. 19.3-107.0.
16-18. — *Interporopollenites vancampoae* n. fsp., prep. Preza—III—7; cross-table No. 15.2/121.1.
19-21. — *Interporopollenites vancampoae* b. fsp., prep. Preza—III—3; cross-table M
19-21. — *Interporopollenites vancampoae* n. fsp., prep. Preza—III—3; cross-table No. 11.9/115.9.
22-24. — *Interporopollenites proporus* W . and K . 1953, prep. Aveiro—4; cross-table No. 18.5/109.4.
25-27. — *Interporopollenites ornatus* n. fsp., prep. Preza—III—7; cross-table No. 15.2/121.2
M: × 1000

Plate 1



Presence: Upper Cretaceous Period, Santon/Campan: Arada, Aveiro, Preza.

1.2. *Interporopollenites endotriangulus* HEGEDŰS, KEDVES and PÁRDUTZ 1972 (Plate 1, 7—9).

Presence: Upper Cretaceous Period, Santon/Campan: Aveiro. It is interesting that, apart from the typical habitat, it has not been found anywhere else as yet.

1.3. *Interporopollenites subgranulosus* n. fsp. (Plate 1, 10—12).

Diagnosis

The equatorial contour is triangular with convex sides. The angles of the pollen grain, particularly in the germinal region, are strongly prominent, and more or less semicircular. The surface is smooth, without decoration. The exopores are more or less elliptical, with a maximum size of 3.4—4 μ. The extragerminal exine is 0.6 μ thick its three layers being by and large of equal thickness. The foot layer is not separated from the columella layer; the endannulus is 2.4—2.6 μ thick. The components of the columella layer are comparatively large and on the whole surface of the pollen grain there is a marked intragranular structure. At the angles of the pollen grain, the columella layer becomes many times thicker. The endogerminalia is wide, with a diameter of 7 μ or so.

Maximum size: 27 μ, in further specimens 26—32 μ.

Holotype: Plate 1, 10—12, prep. Preza—III—18; cross-table No. 15.8 107.4.

Locus typicus: Preza, sediments from the Upper Cretaceous Period.

Stratum typicum: Carbonic clay.

Derivatio nominis: From the structure of the exine.

Differential diagnosis: It can be distinguished well from *I. initium* (PF. 1953a) PF. 1953b by means of its larger size, the exine structure and the prominent germinal region.

Presence: Upper Cretaceous Period, Santon/Campan: Aveiro, Preza.

1.4. *Interporopollenites vancampoae* n. fsp. (Plate 1, 13—21).

Diagnosis

The equatorial contour is triangular, with convex or concave sides; at the angles the germinal region is strongly prominent in the form of a circle. Both surfaces are smooth. The exopores are approximately circular, their diameters being about 2 μ. The extragerminal exine is 1.5 μ thick, and has three layers (tectum, columella layer and foot layer) which are by and large equal. In the germinal region, and primarily at the angles, the columella layer becomes extremely thick, the thickness of this one layer exceeding 2 μ. The endannulus is about 3 μ thick, and the endopore 2 μ wide.

Maximum size: 16 μ, in further specimens 14—20 μ.

Holotype: Plate 1, 13—15, prep. Preza—III—6; cross-table No. 19.3/107.0.

Locus typicus: Preza, sediments from the Upper Cretaceous Period.

Stratum typicum: Carbonic clay.

Derivatio nominis: From Dr. M. Van Campo.

Differential diagnosis: It is distinguished from *I. initium* (PF. 1953a) PF. 1953b, as well as from *I. subgranulosus* n. fsp. by its smaller size, the separate germinal regions, and thinner extragerminal exine.

Presence: Upper Cretaceous Period, Santon/Campan: Aveiro, Preza.

2.1. *Interporopollenites proporus* WEYL. and KRIEG. 1953 (Plate 1, 22—24).

Presence: Upper Cretaceous Period, Santon/Campan: Aveiro, Preza.

2.2. *Interporopollenites ornatus* n. fsp. (Plate 1, 25—27; Plate 2, 1—3).

Diagnosis

The equatorial contour is triangular, with mildly convex sides. The germinal region is only slightly prominent, mainly at the angles of the pollen grain. The surface is granulate or finely rugulate, and the size of the sculpture components is 1—1.5 μ. The exopores are drop-shaped, generally elongated in the radial direction, their average size being 3×2 μ. Round the exogerminaliae, there is an ectexine thickening about 1.5 μ wide, an annulus, with a granulate surface of undulatory rim. The exine is usually 2.5 μ thick, and three—layered. The tectum is very thin (V=1/2-3/1). The foot layer becomes thick centripetally in the germinal region, and there is an endannulus about 3 μ thick. The columella layer is not separated from the foot layer, and there is no vestibulum. At the angle, the columella layer is only a little thicker than in the extragerminal region. The endopore is about 1 μ wide.

Maximum size: 21 μ, in further specimens 19—23 μ.
Holotype: Plate 1, 25—27, prep. Preza—III—7; cross-table No. 15.2/121.2.
Locus typicus: Preza, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From its decorated surface.
Differential diagnosis: It is well-distinguished from the other Interporopollenites fsp. by its characteristic sculpture, smaller size, and relatively thick exine.
Presence: Upper Cretaceous Period, Santon Campan: Arada, Preza.

2.3. *Interporopollenites rugulatus* n. fsp. (Plate 2., 4—9).

Diagnosis

The equatorial contour is triangular, with straight, mildly convex or concave sides. Both surfaces are flat and decorated with 1—4 μ wide rugulate elements. The exopores are elliptical, their average size being 2×3 μ. The extragerminal exine is 1 μ thick, and three-layered; the tectum, columella layer, and foot layer are of equal thickness. At the angles, the columella layer becomes a little thicker, and the two external layers of the ectexine are 1 μ thick here too. The foot layer in the germinal region becomes very thick. The endannulus is wedge-shaped and mostly 2 μ thick. The endopore is 3 μ in diameter, widening out centrifugally.

Maximum size: 26 μ, in further specimens 24—30 μ.
Holotype: Plate 2, 4—6, prep. Preza—III—5; cross-table No. 15.9/104.3.
Locus typicus: Preza, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From the characteristic sculpture of the pollen grain.
Differential diagnosis: It differs from *I. ornatus* n. fsp. in its characteristic sculpture and in having no decoration round the endopores.
Presence: Upper Cretaceous Period, Santon/Campan: Aveiro, Preza.

2.4. *Interporopollenites weylandi* n. fsp. (Plate 2., 10—12).

Diagnosis

The middle part of the pollen body is approximately circular. The germinal region is circularly very prominent. On both surfaces, the exine part that connects the germinal regions is more or less prominent, with a Y shape. In the prominent part, the exine is somewhat thicker than in other parts of the pollen grain. The ornamentation is rugulate. The extragerminal exine on the equator is extremely thin, about 0.4—0.5 μ. Often even the triple division of the ectexine cannot be recognized.

The surface is smooth. The exospores are generally elliptical, their maximum size being about 4—6 μ. In the germinal region, the tectum and columella layer of the ectexine are considerably thicker than the extragerminal ectexine: 1.3—1.5 μ. The separation of the tectum and columella layer is marked in this region. The vestibulum is comparatively large, the foot layer curving, often having in its middle a hardly observable endogerminalia, with an about 3 μ diameter. There is no endannulus.
Maximum size: 28 μ, in further specimens 24—30 μ.
Holotype: Plate 2, 10—12, prep. Preza—III—19; cross-table No. 8.1/109.0.
Locus typicus: Preza, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From Prof. Dr. R. H. Weyland, one of the describers of the genus.
Differential diagnosis: It is well-distinguished from the other pollen grains by its vestibulum and characteristic thickening.
Presence: Upper Cretacous Period, Santon/Campan: Preza.

2.5. *Interporopollenites nagyae* n. fsp. (Plate 2, 13—18).

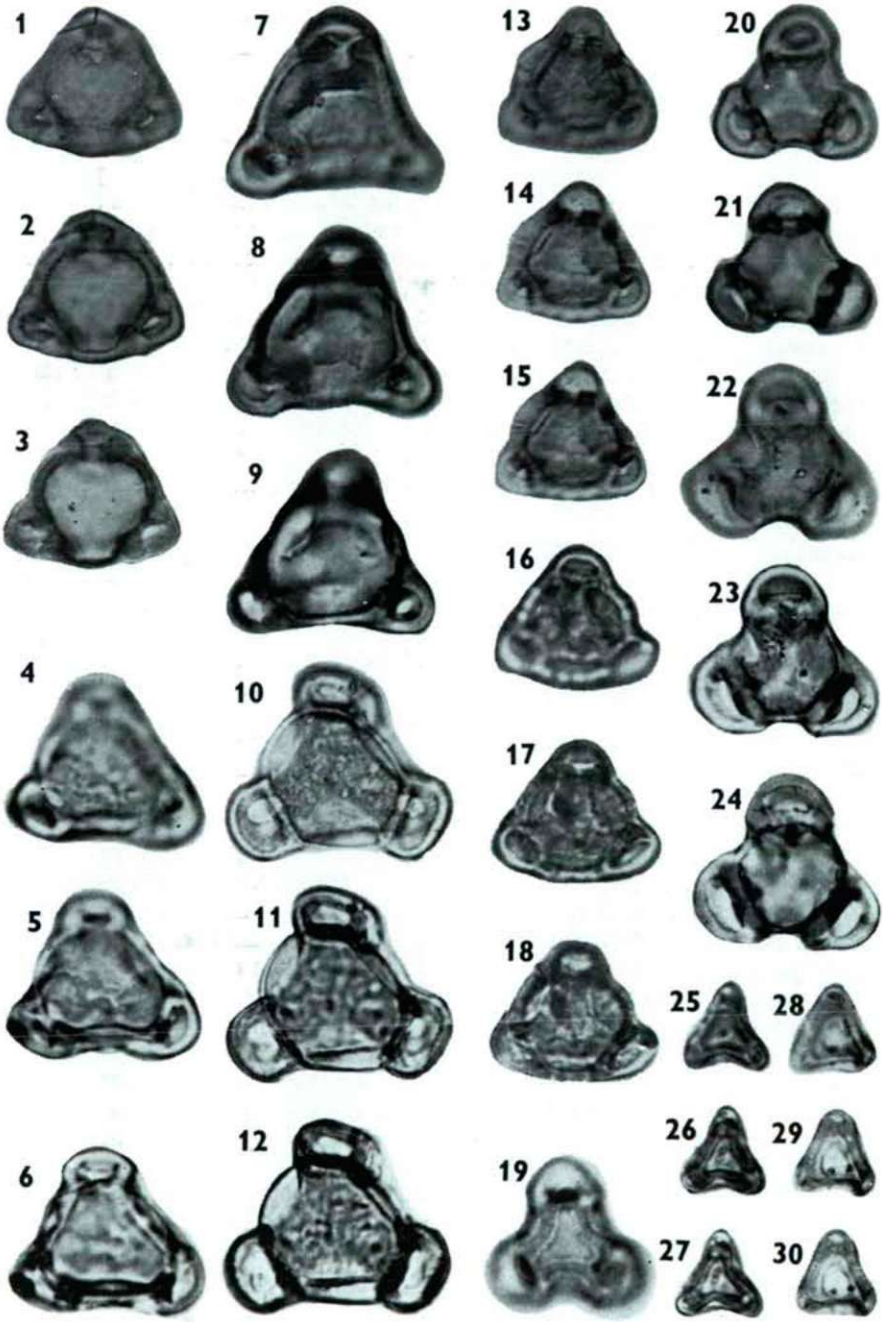
Diagnosis

The equatorial contour is triangular, with convex or undulatory sides. The characteristic of the form-species is given by the comparatively thick ectexine, with verrucate-rugulate decoration and, on both surfaces, with 4 μ wide ectexine thickenings (arci) passing from angle to angle, their rim being undulatory, leaving in the middle a thinner ectexine part that is by and large triangular. The extragerminal exine is generally 2.5 μ thick. Of the three layers, the foot layer is the thinnest, while the tectum and the columella layer are by and large of equal thickness. The columella layer is a little separated from the thickened foot layer. In contrast to the other form-species, in the angles the columella layer grows a little narrow. The combined thickness of it and the tectum is only 1 μ. The endannulus is 1.5 μ thick, and the diameter of the endogerminalia is 3 μ or so.
Maximum size: 20 μ, in further specimens 18—22 μ.
Holotype: Plate 2, 13—15, prep. Preza—III—8; cross-table No. 14.0/106.9.
Locus typicus: Preza, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From Dr. E. Nagy.

Plate 2. 1-3. — *Interporopollenites ornatus* n. fsp., prep. Preza—III—17; cross-table No. 17.2/108.8.
4-6. — *Interporopollenites rugulatus* n. fsp., prep. Preza—III—5; cross-table No. 21.2/107.0.
10-12. — *Interporopollenites weylandi* n. fsp., prep. Preza—III—19; cross-table No. 8.1/109.0.
13-15. — *Interporopollenites nagyae* n. fsp., prep. Preza—8; cross-table No. 14.0/106.9.
16-18. — *Interporopollenites nagyae* n. fsp., prep. Preza—III—1; cross-table No. 17.0/105.6
19-21. — *Interporopollenites zaklinskaiae* n. fsp., prep. Preza—III—3; cross-table No. 8.2/116.2.
22-24. — *Interporopollenites zaklinskaiae* n. fsp., prep. Preza—III—11; cross-table No. 5.6/104.5.
25-27. — *Interporopollenites microporus* n. fsp., prep. Aveiro—8; cross-table No.4.2-104.9.
28-30. — *Interporopollenites microporus* n. fsp., prep. Preza—III—8( cross-table No. 9.0-118.1.

M: ×1000

Plate 2


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

Differential diagnosis: It is distinguished from the other pollen grains of the form-genus by its characteristic decorative elements.
Presence: Upper Cretaceous Period, Santon/Campan: Arada, Preza.

2.6. *Interpropollenites zaklinskaiae* n. fsp. (Plate 2, 19—24).

Diagnosis

The equatorial contour is triangular, with straight or convex sides, and with a half-ball shaped germinal region which is strongly separated from the pollen body. On both proximal and distal surfaces there is a characteristic thickened arcus, about 2.4—3 μ wide, from angle to angle, that is perforated finely, and contains in the middle of both poles a by and large triangular space. The level of the exopores is in the germinal part, below the exine part mentioned. The arci partly convert up the ellipsoidal exopores whose maximum size is about 5 μ. The extragerminal exine on the equator is extremely thin; it is always below 0.5 μ: 0.3—0.4 μ. The surface is smooth. The triple division of the exine cannot be recognized exactly because of its being extremely thin. In the germinal part, however, the exine is very thick. The foot layer forms a 2.4 μ thick endannulus. There is no vestibulum. The tectum is very narrow. At the angles of the pollen, a large part of the ectexine is given by the columella layer, whose structure, too, can be recognized a little. The endogerminalia is 2.4 μ wide.

Maximum size: 24 μ, in further specimens 20—30 μ.
Holotype: Plate 2, 19—21, prep. Preza—III—3; cross-table No. 8.2/116.2.
Locus typicus: Preza, layers from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From Prof. Dr. Zaklinskaia, E.D.
Differential diagnosis: Its prominent germinal region and the decorated surface of the arci differ from those of *I. nagyae* n. fsp.
Presence: Upper Cretaceous Period, Santon/Campan: Aveiro, Preza.

2.7. *Interporopollenites microporus* n. fsp. (Plate 2, 25—30).

Dignosis

The equatorial contour is triangular, with straight or mildly concave sides. The germinal region is not prominent. On both surfaces, the ectexine grows significantly thick arci, surrounding a triangular space in the middle. It does not surround the angles but is united before them. Along the sides, the surface formations are about 1 μ wide and approximately 2 μ near the poles. The exopores are remarkably small, their maximum diameter not exceeding 0.5 μ; their shape is circular. Owing to the characteristic formations of the pollen grains, the structure of the equatorial exine can be studied only with difficulty. The extragerminal exine is generally 1 μ thick, the three layers being by and large of equal thickness. The foot layer in the germinal region becomes characteristically thick, about 1 μ, forming a more or less wedge-shaped endannulus. The endopore is comparatively wide, its diameter being about 2 μ.
Maximum size: 12 μ, in further specimens 10—14 μ.
Holotype: Plate 2, 25—27, prep. Aveiro—8, cross-table No. 4.2/103.9.
Locus typicus: Aveiro, layers from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From its small exopores.

Differential diagnosis: It is distinguished from the similar pollen grains by its small size and characteristic surface decoration.

Presence: Upper Cretaceous Period, Santon/Campan: Aveiro, Preza.

The heteropolar pollen grains are similarly divided two subgroups: (1) only one surface of the pollen grains is decorated, while the other is smooth; (2) both surfaces are decorated, the decoration being different.

3.1. *Interporopollenites nimbus* PF. 1953b (Plate 3, 1—3).

Presence: Upper Cretaceous Period, Santon/Campan: Arada, Aveiro.

3.2. *Interporopollenites plicatus* n. fsp. (Plate 3, 4—6).

Diagnosis

The equatorial contour is triangular, with mildly convex or concave sides. On one of the surfaces there are definite, narrow arci, passing from angle to angle. The exopores are mostly radially-oriented ellipsoidal or drop-shaped, with an average size of $3.4 \times 2\,\mu$. The other surface is smooth. In the extragerminal region it is thin, usually $1\,\mu$, the tectum, columella layer, and foot layer being by and large of equal thickness. At the angles of the pollen grain, the columella layer becomes thick; the combined thickness of the two external layers is about $1.5\,\mu$. The endannulus is small, often hardly observable. It is $2\,\mu$ thick.

Maximum size: $35\,\mu$, in further specimens $32—40\,\mu$.
Holotype: Plate 3, 4—6; prep. Arada—8—1, cross-table No. 19.3/119.4.
Locus typicus: Arada, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From the characteristic plicae-like arci.
Differential diagnosis: It is well distinguished from *I. nimbus* PF. 1953b by its much larger size and its plicae-like arci, and by that the arci surround a thin-walled triangular space. In *I. nimbus* PF. 1953b the arci are Y-shaped.
Presence: Upper Cretaceous Period, Santon/Campan: Arada.

3.3. *Interporopollenites triangulus* n. fsp. (Plate 3, 7—9).

Diagnosis

The equatorial contour is triangular, with straight sides and angles rounded off only a little. It is a heteropolar pollen, but this is only slightly marked. On one side, at the pole, there is a more or less triangular exine attenuation. There are no further differentiations in the pollen grain. The exopores are generally radially-oriented, and ellipsoidal or drop-shaped. Their lenght is about $3.4\,\mu$, and their width $1\,\mu$ or so. The extragerminal exine on the equator is $1.8\,\mu$ thick. The foot layer reaches about half the complete thickness of the ectexine; the tectum is a little thicker than the columella layer. There is no vestibulum. The endannulus is generally $3\,\mu$ thick. In the apex, the tectum and foot layer are about $1\,\mu$, and the diameter of endopore is similarly $1\,\mu$.

Maximum size: $17\,\mu$, in further specimens $15—20\,\mu$.
Holotype: Plate 3, 7—9, prep. Preza—III—2; cross-table No. 21.0/109.4.
Locus typicus: Preza, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From its characteristic triangular contour.

Differential diagnosis: It is distinguished from *I. nimbus* PF. 1953b, which is similar in size to it, by the shape of the exopores and the exine-attenuation at one of the pollen.
Presence: Upper Cretaceous Period, Santon/ Campan: Preza.

3.4. *Interporopollenites thomsoni* n. fsp. (Plate 3, 13—15).

Diagnosis

The equatorial contour is triangular, with convex sides. The germinal region is a little prominent and on the surface, round the exospores, there is a well-definiable annulus about 4 μ wide; the other surface is smooth, without any particular formation. The exopores have 3.5—4 μ diameters and are circular or ellipsoidal. The extragerminal exine in the equatorial region is generally about 1 μ thick, and the tectum, columella layer, and foot layer are by and large equally thick. In the germinal region, the thickened foot layer links up closely with the columella layer. The endannulus is characteristically wedge-shaped, and 5 μ thick. At the angle of the pollen grain the germinal tectum and columella layer are comparatively thin.
Maximum size: 30 μ, in further specimens 27—32 μ.
Holotype: Plate 3, 13—15, prep. Preza—III—22; cross-table No. 20.9/110.9.
Locus typicus: Preza, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: In memory of Prof. Dr. P. W. Thomson.
Differential diagnosis: It is distinguished within the form-genus by means of its characteristic annuli and by the absence of arci.
Presence: Upper Cretaceous Period, Santon/Campan: Preza.

3.5. *Interporopollenites krempi* n. fsp. (Plate 3, 16—21).

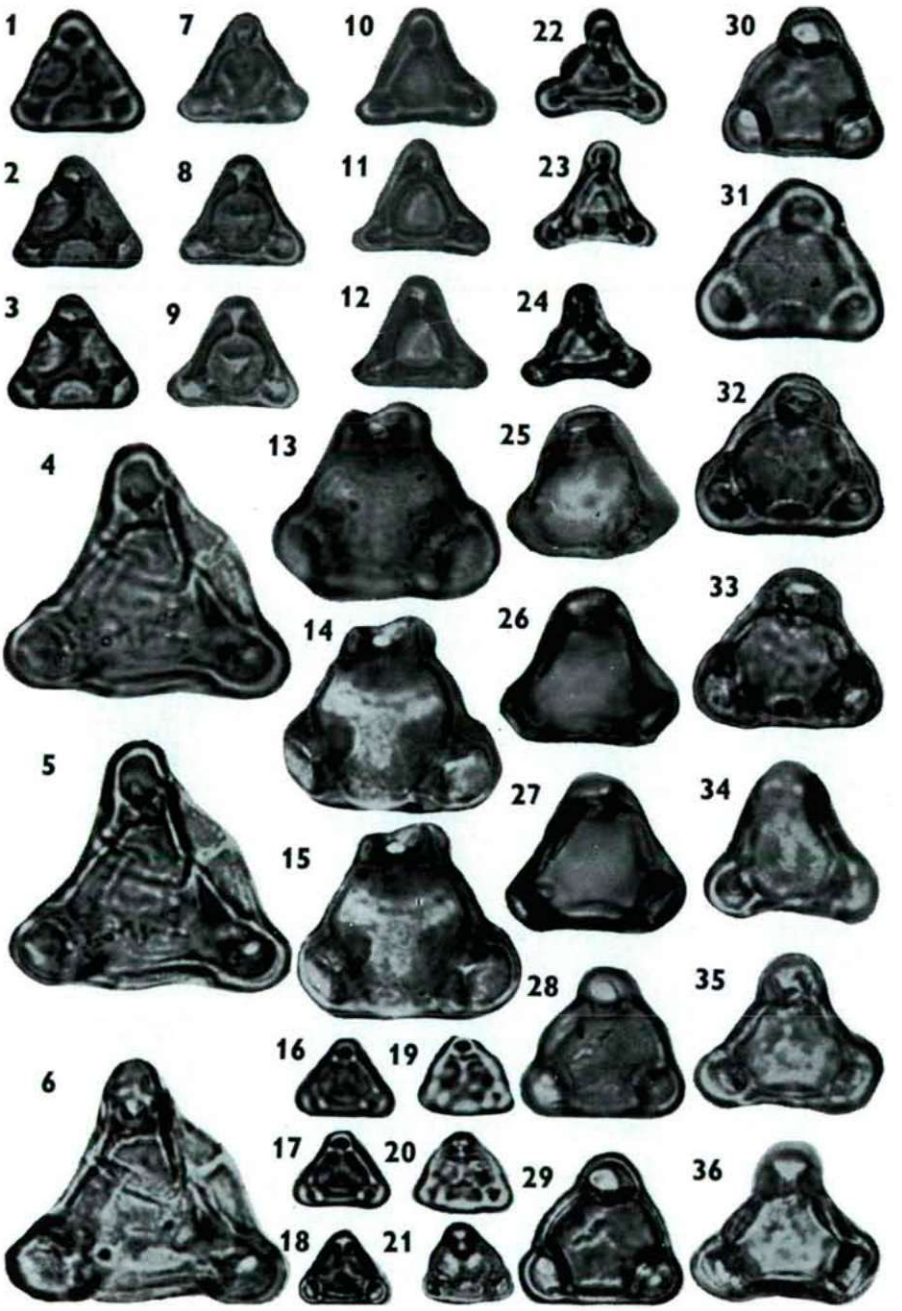
Dignosis

The equatorial contour is triangular, generally with straight sides. On one of the surfaces, there are Y-shaped arci. This widens out a little at the angles; similarly, as for *I. nimbus,* the other surface is smooth. The exopores are small and circular, and their diameter does not generally exceed 0.5 μ. The extragerminal exine is thin, generally 0.6 μ thick; the tectum, columella layer, and foot layer are by and large of equal thickness. In the germinal region, the columella layer is only a little thicker

Plate 3. 1-3. — *Interporopollenites nimbus* P . 1953b, prep. Aveiro—4; cross-table No. 18.5/109.4
4-6. — *Interporopollenites plicatus* n. fsp., prep. Arada—8—1; crossttable No.19.3/119.4
7-9. — *Interporopollenites triangulus* n. fsp., prep. Preza—III—2; cross-table No. 21.0/109.4.
10-12. — *Interporopollenites guineti* n. fsp. Preza—III—1; cross-table No. 9.3/120.9
13-15. — *Interporopollenites thomsoni* n. fsp., prep. Preza—III—22; cross-table No. 20.9/110.9.
16-18. — *Interporopollenites krempi* n. fsp., prep. Aveiro—2; cross-table No. 8.1/118.9.
19-21. — *Interporopollenites krmpi* n. fsp. prep., Aveiro—8; cross-table No. 15.0/11.00
22-24. — *Interporopollenites guineti* n. fsp., prep. Aveiro—2; cross-table No. 6.8/110.2
25-27. — *Interporopollenites concavus* n. fsp., prep. Preza—III—10; cross-table No. 8.8/111.5.
28-30. — *Interporopollenites stanleyi* n. fsp., prep. Preza—III—24; cross-table No. 16.2/114.1.
31-33. — *Interporopollenites stanleyi* n. fsp., prep. Aveiro—23; cross-table No. 6.5/117.7.
34-36. — *Interporopollenites stanleyi* n. fsp. p,rep. Preza—III—2; cross-table No. 16.2/117.4.

M: ×1000

Plate 3


1
7
10
22
30
2
8
11
23
31
3
9
12
24
32
4
13
25
33
14
26
5
27
34
15
28
35
16
19
6
17
20
29
36
18
21

than in the extragerminal region. There is a small vestibulum and a similarly smaii, drop-shaped endannulus. The diameter of the endopore is about 1.5 μ.
Maximum size: 11 μ, in further specimens 9—14 μ.
Holotype: Plate 3, 16—18; prep. Aveiro—2, cross-table No. 8.1/118.9.
Locus typicus: Aveiro, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From Prof. Dr. G. O. W. Kremp.
Differential diagnosis: It is well-distinguished from *I. nimbus* PF. 1953b mainly by its smaller size.
Presence: Upper Cretaceous Period, Santon/Campan: Aveiro.

3.6. *Interporopollenites guineti* n. fsp. (Plate 3, 10—12, 22—24).

Diagnosis

The equatorial contour is triangular, with concave sides; the angles are rounded, and the germinal region is a little prominent. On the one side there are very significant arci that are generally 2 μ wide and leave a regular triangular part free in the middle. The exopores are more or less drop-shaped, their length being about 2 μ and their width 0.7 μ or so. The other surface is smooth. The extragerminal exine on the equator is generally 1 μ thick; the tectum, columella layer, and foot layer are by and large no surface decorating elements or tectum perforations can be recognized. The foot layer in the germinal region does not become separated from the columella layer and does not form any endannulus. The endogerminaliae are about 1.5 μ thick. The columella layer at the angles becomes very thick.
Maximum size: Plate III, 10—12, prep. Preza—III—1, cross-table No. 9.3/120.9.
Locus typicus: Aveiro, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From Dr. Ph. Guinet.
Differential diagnosis: It is well-distinguished from the former form-species by the arci, which are parallel with the equator of one of the surfaces, and also by the larger size.
Presence: Upper Cretaceous Period, Santon/Campan: Aveiro.

3.7. *Interporopollenites concavus* n. fsp. (Plate 3, 25—27).

Diagnosis

The equatorial contour is triangular, with concave sides; the angles are widely rounded, are sometimes nearly angular. On the sculptured surface, there is a characteristic exine thickening, arci, connecting the sides of the germinaliae of the pollen grain and surrounding half the exopores too. The other surface is smooth. The exopores are mostly ellipsoidal, with a maximum size of 2—2.5 μ. The extragerminal exine on the equator is 1.5 μ thick, the tectum, columella layer, and foot layer being generally of equal thickness. In the germinal region, the thickening foot layer is not separated from the columella layer. There is no vestibulum. The endannulus is more or less wedge-shaped, its maximum width being 2 μ. In the poles of the pollen grain the columella layer becomes thick, together with the tectum being as thick as the extragerminal exine. The endogerminalia is wide, with a diameter of about 4 μ.
Maximum size: 23 μ, in further specimens 20—25 μ.
Holotype: Plate 3, 25—27, prep. Preza—III—10, cross-table No. 8.8/111.5.
Locus typicus: Preza, sediments from the Upper Cretaceous Period.

Stratum typicum: Carbonic clay.
Derivatio nominis: From its concave contour.
Differential diagnosis: The surface formation of one side, and the size and the form of the pores are good distinguishing marks within the form-genus.
Presence: Upper Cretaceous Period, Santon/Campan: Preza.

3.8. *Interporopollenites stanleyi* n. fsp. (Plate 3, 28—36).

Diagnosis

The equatorial contour is triangular, with mildly convex sides, and the germinal region is a little prominent. One of the sides is smooth, without any surface formations, On the other side, there are characteristic arci, about 4 μ wide, ribbon-shaped, and reaching from pore to pore. In the middle is a regular triangular part thin-walled. In the middle is a regular triangular part thin-walled. The exopores are elliptical or circular, oriented in the radial direction, their maximum size being about 3 μ. The extragerminal equatorial ectexine is generally 1 μ thick, the tectum, columella layer, and foot layer are largely of the same thickness. In the germinal region, the foot layer is a little separated from the columella layer; there is a small vestibulum, which thickened clublike, creates an endannulus that is generally 1.3 μ thick. At the angles, the columella layer becomes very thick; it is about the quadruple of that in the equatorial region. The endopores are 3.5—3.8 μ wide.

Maximum size: 22 μ, in further specimens 20—25 μ.
Holotype: Plate 3, 28—30, prep. Preza—III—24; cross-table No. 16.2/114.1.
Locus typicus: Preza, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From Prof. Dr. E. A. Stanley.
Differential diagnosis: It is distinguished from *I. guineti* n. fsp. by its wide surface formations.
Presence: Upper Cretaceous Period, Santon/Campan: Preza.

4.1. *Interporopollenites pflugi* n. fsp. (Plate 4, 1—6).

Diagnosis

The equatorial contour is triangular, generally with straight sides. The angles are rounded, the germinal region being a little prominent. Both surfaces, are decorated. On the one side, beside the Y-shaped wide arcus there is a characteristic ectexine attenuation parallel to the sides and having the shape of a split or narrow crescent, about 5—6.5 μ long and 1—1.5 μ wide. The exopores are mostly radially-oriented ellipsoidal, measuring 1—1.5×3—4 μ. The surface including that of the arci seems to be smooth and perforation-free. On the other side, the exopores correspond to the former ones with respect to size and arrangement, but at the pole there is a largely circular, finely-granular ectexine thickening 4 μ wide. The exopores are surrounded in the centripetal direction by a similar, finely-granular ectexine thickening that seems to be smooth at the apex of the pollen. Its width is about 4 μ. The exine consist only of ectexine, being 1.5 μ extragerminally; the tectum, columella layer, and foot layer are by and large equally thick. The foot layer in the germinal region becomes very thick, forming a more or less club-shaped endannulus. The germinal columella layer becomes similarly very thick as compared with the extragerminal region. There is no vestibulum; the endogereminalia is generally 4 μ wide.

Maximum size: 26 μ, in further specimens 24—32 μ.

Holotype: Plate 4, 1—3, prep. Arada—8—7; cross-table No. 14.5/111.2.
Locus typicus: Arada, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From Prof. Dr. H. PFLUG, the elaborator of the Normapolles stemma taxonomy.
Differential diagnosis: The comparatively strong and different decorations of both surfaces are well-distinguished within the form-genus.
Presence: Upper Cretaceous Period, Santon/Campan: Arada.

4.2. *Interporopollenites prezaensis* n. fsp. (Plate 4, 7—15).

Diagnosis

The equatorial contour is triangular, with convex sides; the germinal region is semicircularly prominent. On one of the surfaces, there is a characteristic Y-like arcus. This is given by the thicker part of the ectexine, the rim of which is undulatory and strongly enlarged near the angles of the pollen grain. The exopores are more or less triangular or ellipsoidal, oriented in the tangential direction, their size being about 4—4.5 μ. On the other surface, the germinal foramina are surrounded essentially by flat, wide, oculus-like annuli. On this surface, the exopores are generally ellipsoidal, oriented radially, their maximum size being 5—6 μ. The extragerminal exine on the equator is about 2 μ thick; the tectum is narrow, and its thickness cannot be established light-microscopically. The perforations of the tectum can be seen well with a light-microscope. On the surface, no decoration is to be observed. The columella layer is well separated, but a detailed morphology of its elements cannot be recognized light-microscopically. Of the three layers, in the extragerminal region on the equator the foot layer is the thickest one. At the vestibulum, the columella layer is the thickest one. At the vestibulum, the columella layer becomes thick; round the endopores the foot layer does the same, forming characteristic endannuli. The endopores have diameters of about 4 μ.
Maximum size: 43 μ, in further specimens 40—48 μ.
Holotype: Plate 4, 7—9, prep. Preza—III—2; cross-table No. 11.4/115.8.
Locus typicus: Preza, sediments from the Upper Cretaceous Period.
Stra um typicum: Carbonic clay.
Derivatio nominis: From Preza, the typical habitat.
Differential diagnosis: It is distinguished well from *I. pflugi* n. fsp. by its larger size and the decoration of its surface.
Presence: Upper Cretaceous Period, Santon/Campan: Preza.

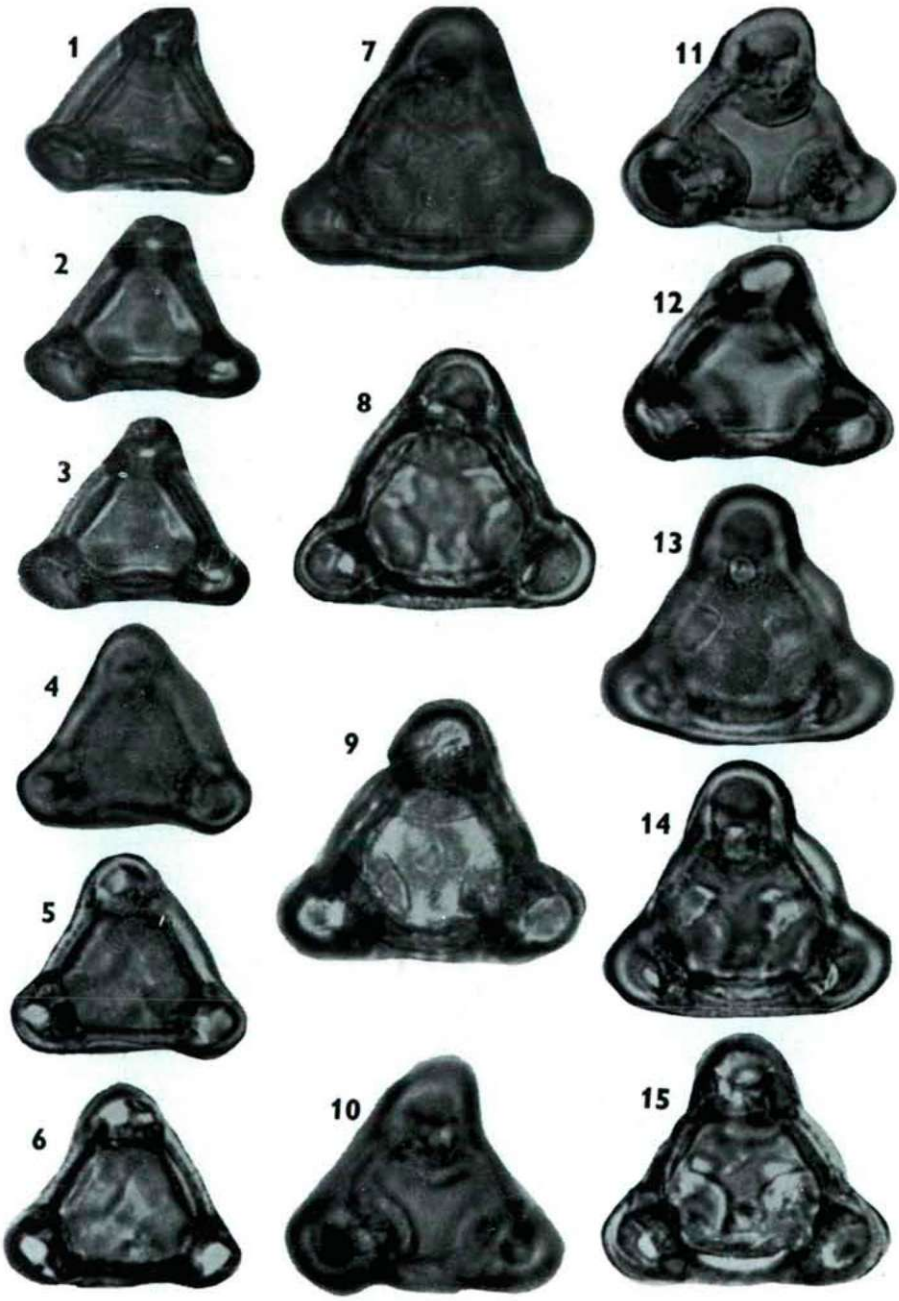
4.3. *Interporopollenites heteropolatus* n. fsp. (Plate 5, 1—6).

Diagnosis

The equatorial contour is triangular, generally with straight sides. In one of the angles, there is an ectexine attenuation of about 4 μ diameter. On both surfaces,

Plate 4. 1-3. — *Interporopollenites pflugi-n.* fsp., prep. Arada—8—7; cross-table No. 14.5/111.2.
4-6. — *Interporopollenites pflugi-n.* fsp., prep. Preza—III—4; cross-table No. 19.9/116.4
7-9. — *Interporopollenites prezaensis* n. fsp., prep. Preza—III—2; cross-table No. 11.4/115.8.
10-12. — *Interporopollenites prezaensis* n. fsp., prep. Preza—III—5; cross-table No. 10.6/115.8.
13-15. — *Interporopollenites prezaensis* n. fsp., prep. Preza—III—3; cross-table No. 18.8/116.5.
M: ×1000

Plate 4



there are characteristic arci, mostly surrounding a triangular space and uniting circularly round the exopores. The exopores can sometimes be observed with difficulty; they are small and circular, their maximum size being about 1 μ. The extragerminal exine is very thin, generally 0,6 μ; the tectum, columella layer and foot layer are equally thick. The foot layer becomes only a little thicker in the germinal region; the endannulus is drop-shaped, its thickness not generally reaching 1 μ. There is a small vestibulum; the diameter of the endopore is 1.7 μ.
Maximum size: 15 μ, in further specimens 12—18 μ.
Holotype: Plate 5, 1—3, prep. Aveirc—14; cross-table No. 8.2/112.8.
Locus typicus: Aveiro, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From the two different surfaces of the pollen grain.
Differential diagnosis: It is well-distinguished from pollen grains of similar type by its smaller size.
Presence: Upper Cretaceous Period, Santon/Campan: Aveiro.

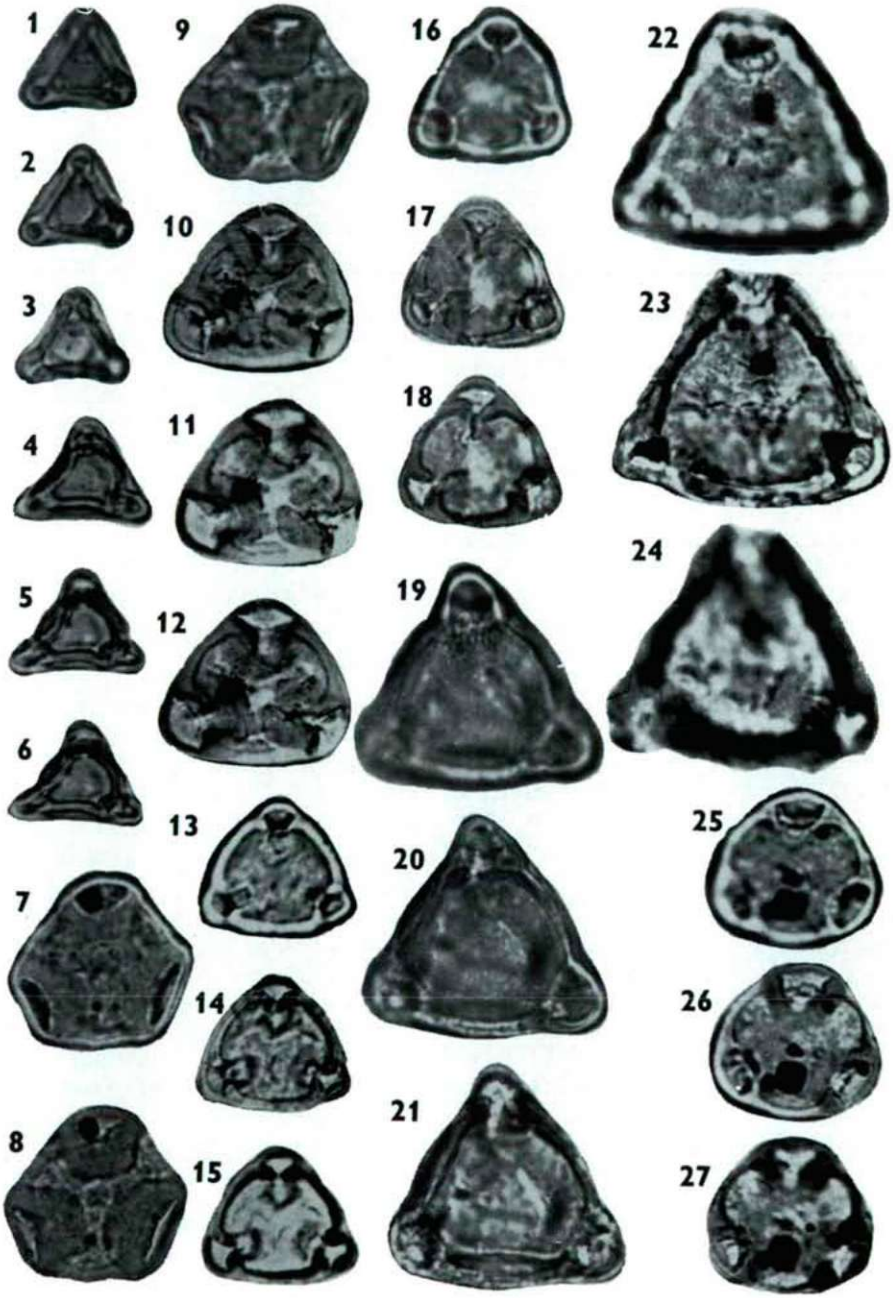
4.4. *Interporopollenites aveiroensis* n. fsp. (Plate 5, 7—9, cf. 10—12).

Diagnosis

The contour is more or less hexagonal, with rounded angles. In the middle of one the poles, there is a small Y-shaped tetrad mark. In the germinal region, round the exopores, the ectexine becomes somewhat thicker and from angle to angle there is a wide Y-shaped formation. The rim of the thickening is undulatory, and round the tetrad-mark the exine is a little thicker. On the other side, the thickening extends only over the surroundings of the exogerminaliae, centripetally from the equator being about 5 times wider than along the equatorial contour. This thickening, that is essentially an annulus, is reminiscent of the oculus. The exogerminaliae grow longer in the tangential direction; they are split-like, and 10×2—3 μ in size. The surface of the pollen grain is smooth or a little scabrate. The extragerminal exine is about 2 μ thick. The well-separated tectum and columella layer are more or less of the same thickness. In the germinal region, the columella layer becomes somewhat thick. The vestibulum is marked and the foot layer becomes very thick. There is an endannulus of centripetal direction. The endopores are by and large 3 μ wide.
Maximum size: 25 μ, in further specimens 22—30 μ.
Holotype: Plate 5, 7—9, prep. Aveiro—15; cross-table No. 5.9/101.7.
Locus typicus: Aveiro, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From Aveiro, the habitat of the typical specimen.
Differential diagnosis: Its essential morphological marks are the same as those

Plate 5. 1-3. — *Interporopollenites heteropolatus* n. fsp., prep. Averio—14; cross-table No. 8.2/112.8.
4-6. — *Interporopollenites heteropolatus* n. fsp., prep. Averio—8; cross-table No. 14.6/111.7.
7-9. — *Interporopollenites aveiroensis* n. fsp., prep.Aveiro—15; cross-table No.5.9/101.7.
10-12. — *Interporopollenites* cf. *aveiroensis* n. fsp., prep. Aveiro—1; cross-table No. 10.1/114.9.
13-15. — *Interporopollenites dinizae* n. fsp., prep. Aveiro—2; cross-table No. 15.5/107.2.
16-18. — *Interporopollenites dinizae* n. fsp., prep. Preza—III—2; cross-table No. 7.9/105.0.
22-24. — *Interporopollenites* cf. *goczani* n. fsp., prep. Aveiro—16; cross-table No. 6.3/130.5
25-27. — *Interporopollenites kriegeri* n. fsp., prep. Aveiro—14; cross-table No. 9.9/108.0.
M: ×1000

Plate 5



of *I. prezaensis* n. fsp. It is well-distinguished primarily by the shape of the exogerminaliae, while in addition the smaller size and the form of the conture are to be mentioned as well.

Presence: Upper Cretaceous Period, Santon/Campan: Aveiro.

4.5. *Interporopollenites dinizae* n. fsp. (Plate 5, 13—18).

Diagnosis

In polar aspect the contour is triangular, with rounded angles and convex sides. The germinal region is not prominent; the surface is smooth, and a thickening can be observed only round the exogerminal pores. This thickening is not the same on both surfaces. The less-developed sculpture is essentially a 1.4—2 μ wide annulus of irregular rim; the more developed one on the other surface similarly surrounds the exogerminal pores, but it also extends towards the pole of the pollen grain and these three annuli nearly or completely unite. The exogerminal pores are located comparatively in the neighbourhood of the centre of the pollen grain; their rim is irregularly undulatory, their shape is mostly radially stretched, and their maximum size is about 4 μ. The extragerminal exine is 1.2—1.5 μ thick, the foot layer being the thickest of the three layers. In the germinal region, the foot layer becomes a little thicker and there is an endannulus curving in towards the pollen centre. The thickness of the endannulus is 2 μ or so, and the width of the endopore is about 1—1.4 μ.

Maximum size: 23 μ, in further specimens 20—27 μ.

Holotype: Plate 5, 13—15, prep. Aveiro—2; cross-table No. 15.5/107.2.

Locus typicus: Aveiro, sediments from the Upper Cretaceous Period.

Stratum typicum: Carbonic clay.

Derivatio nominis: From Dr. F. Diniz who made the material of investigation available to us.

Differential diagnosis: It is well-distinguished from the pollen grains of similar size by its characteristic sculpture.

Presence: Upper Cretaceous Period, Santon/Campan: Preza.

4.6. *Interporopollenites goczani* n. fsp. (Plate 5, 19—21, cf. 22—24).

Diagnosis

The equatorial contour is triangular, with mildly convex or straight sides. The germinal region is not or only a little prominent. On one of the surfaces there are arci about 6 μ wide, and on the poles there is a tirangular-shaped exine part. The exopores are approximately circular or ellipsoidal oriented in the radial direction, with average size 3 μ and respectively 2×4 μ. Round the pores, in the centripetal direction, there is an annulus about 3—4 μ wide which is decorated on both surfaces with granules of finely rugulate elements. The extragerminal exine is about 2 μ thick. The foot layer is the thickest of the three layers, comprising half of the total thickness of the ectexine. The tectum and the columella layer are by and large of equal thickness. At the angles the tectum and the columella layer are 1.5 μ. In the germinal region, the foot layer becomes very thick and forms a maximum 4 μ thick wedge-shaped endannulus. The endopore is wide, its diameter generally being 5 μ.

Maximum size: 32 μ, in further specimens 29—36 μ.

Holotype: Plate 5, 19—21, prep. Preza—III—2; cross-table No. 8.8/120.2.

Locus typicus: Preza, sediments from the Upper Cretaceous Period.

Stratum typicum: Carbonic clay.

Derivatio nominis: From Dr. F. Góczán.
Differential diagnosis: It is well-distinguished from *I. dinizae* n. fsp. by arci on the side, from *I. plicatus* n. fsp. by the shape of the annulus and the ornamentation.
Presence: Upper Cretaceous Period, Santon/Campan: Preza.

4.7. *Interporopollenites kriegeri* n. fsp. (Plate 5, 25—27).

Diagnosis

The equatorial contour is triangular, with rounded angles and convex sides. The germinal region is not prominent from the equatorial amb of the pollen grains. It is characteristic of the surface ornamentation that it is only slightly marked. One of the sides is nearly entirely smooth the exine becoming a little thicker only round the exopores, and a narrow annulus appears. On the other side, there is a Y-shaped arcus with a full width of about 7—10 μ. The exopores are circular or ellipsoidal, their maximum size being 2—3 μ. The thickness of the extragerminal exine is about 1 μ. The tectum, columella layer and foot layer are of almost equal thickness. The foot layer gradually becomes thicker in the direction of the germinal region, forming a wedge-shaped endannulus with maximum thickness of 3 μ. In the germinal region, the foot layer is not separated from the columella layer. The diameter of the endogerminalia is about 5 μ.
Maximum size: 25 μ, in further specimens 22—28 μ.
Holotype: Plate 5, 25—27, prep. Aveiro—14; cross-table No. 9.9/108.0.
Locus typicus: Aveiro, sediments from the Upper Cretaceous Period.
Stratum typicum: Carbonic clay.
Derivatio nominis: From Dr. W. Krieger, one of the authors of the form-genus.
Differential diagnosis: It is well-distinguished within the form-genus by its only slightly-marked decorating elements. It can be distinguished from *I. aveiroensis* n. fsp. by the shape of the annuli.
Presence: Upper Cretaceous Period, Santon/Campan: Aveiro.

## Discussion of results

With regard to the form-species treated in our work it must be stated that this level is extremely rich in these pollen grains, even in an international relation. Notwithstanding that the publications generally indicate one form or two within the Normapolles region in sediments from the Upper Cretaceous Period, they are similarly present in the Palaeocene and Lower Eocene as well, but they can mostly be regarded as incidental elements. A similar facies is locally indicated perhaps by KRUTZSCH's work (1966). A detailed taxonomical elaboration of this has not taken place as yet but we may expect a very interesting comparison from these data. Since the descriptions that may be considered as standard works, our paper is the first to deal with the taxonomy of pollen grains, and thus its character is mainly fundamental and descriptive. It must be pointed out that our knowledge concerning the form-species characters was limited for just that reason. It is possible that several differenciated species will later be found the same. The exact descriptions and the most perfect representations possible, therefore demand very great care. It is to be hoped that before long an opportunity will present itself for the performance of scanning-electronmicroscopic investigations as well; on the above basis, these are especially suitable for making progress in the problems of "species". The point

in question is that there is a facies or level within the Upper Cretaceous Period that can be characterized very well and those predominant formations were the ancient Juglandaceae trees. The presence of the form-species in some habitats is interesting. One form-species occurred in all three habitats, nine in two habitats, and sixteen in one habitat. There were six form-species in Arada, fourteen in Aveiro, and seventeen in Preza; thus Preza is the habitat richest in these pollen grains. Only two occurred conclusion in Arada, six in Aveiro and eight of them in Preza. Withaut drawing too far-reaching from these figures, the following findings may be made: It is possible that one of the peculiarities of the pollen grains of the ancient Amentiflorae is a prodigious richness in species, this is a well-known characteristic of tropical natural history, or the pollen grains of the individual species are rich in forms. In possession of the present data, this problem can only be decided if detailed elaboration takes place both vertically and horizontally within the Normapolles region. As regards the habitats investigated, their full evaluation is possible with the whole of the pollen collections. In this connection we refer to the fact that this place is very interesting and rich in further Normapolles as well; we have already published electronmicroscopic data about a part of these.

## References

GÓCZÁN, F., GROOT, J. J., KRUTZSCH, W. — PACLTOVÁ, B. (1967): Die Gattungen des "*Stemma Normapolles* PFLUG 1953b" (Angiospermae) Neubeschreibung und Revision europäischer Formen (Oberkreide bis Eozän). — Paläont. Abh. B, *2*, 427—633.

HEGEDŰS, M., KEDVES, M., — PÁRDUTZ, Á. (1972): Ultrastructural investigation of Upper Cretaceous Angiosperm exines II. — Acta Biol. Szeged *18*, 55—69.

KEDVES, M. — DINIZ, F. (1967): Quelques types de sporomorphes de sediments crétacés d'Aveiro, Portugal. — Com. dos Servicos Geol. de Portugal *52*, 17—25.

KRUTZSCH, W. (1966): Die sporenstratigraphische Gliederung der Oberkreide im nördlichen Mitteleuropa. Methodische Grundlagen und gegenwärtiger Stand der Untersuchungen. — Abh. Zentr. Geol. Inst. *8*, 79—111.

PFLUG, H. D. (1953): Zur Entstehung und Entwicklung des angiospermiden Pollens in der Erdgeschichte. — Paläontographica B, *95*, 60—171.

THOMSON, P. W. — PFLUG, H. D. (1953): Pollen und Sporen des mitteleuropäischen Tertiärs. — Palaeontographica, B, *94*, 1—138.

WEYLAND, H. — KRIEGER, W. (1953): Die Sporen und Pollen der Aachener Kreide und ihre Bedeutung für die Charakterisierung des mittleren Senons. — Paläontographica B, *95*, 6—29.

Address of the authors:
Dr. M. KEDVES
Dr. MÁRIA HEGEDŰS
Department of Botany
A. J. University, H-6701 Szeged,
P. O. Box 428, Hungary

Acta Biologica Szeged. *21* (1—4), pp. 63—68 (1975)

# ÜBER DIE PFLANZENGEOGRAFISCHE BEDEUTUNG VON DREI NEUEN ARTEN FÜR DIE FLORA DER VOJVODINA

MELANIJA OBRADOVIČ

*Prirodno-matematički fakultet Institut za biologiju, Novi Sad*

(Eingegangen am 27. Februar 1975)

## Einleitung

Während der letzten Jahre hatte ich Gelegenheit, das Vorkommen gewisser Pflanzenarten in der Flora der Vojvodina wahrzunehmen. Durch die Feststellung ihrer systematichen und pflanzengeografischen Zugehörigkeit kam ich zum Schluß, daß diese Arten von gewisser Bedeutung für unsere Flora sind. Diese Arten sind: *Taeniatherum asperum* NEVSKI, *Ranunculus millefoliatus* VAHL. und *Doronicum orientale* HOFFM.

## Analyse

*Taeniatherum asperum* NEVSKI.
*Elymus asper* (SIMK.) RAND.—MAZ.

Diese Pflanze aus der Familie der Gramineae ist eine pontisch-balkanische Art, die nach JÁVORKA (1925) auf der Krim und dem Balkan vorkommt. Als eine thermophile Art von kontinentaler Wesenheit kommt sie auch in der Pannonischen Tiefebene in deren warmen flachlandähnlichen Regionen vor. Laut derselben Quelle, so in KD. (Szentendre, Pomáz, Gellérthegy und Soroksár—Taksony), in A. (Tiszabő), Ald. (Orsova).

Bei uns in der Vojvodina wurde diese Art bis jetzt noch nirgends aufgefunden, worauf sich auf Grund der älteren und neueren Literatur schließen läßt. Dies kommt davon, daß es sich um eine sehr seltene Pflanzenart handelt, was sowohl die angeführten literarischen Quellen bezeugen, wie auch die Tatsache, daß wir sie während unserer vieljährigen Forschungen erst in der letzten Zeit und nur an einer einzigen Stelle aufgefunden haben. Die Pflanze wurde auf dem Banstol in der Fruška Gora, als Bestandteil der Vegetation einer Bergwiese mit ausgeprägten steppenartigen Elementen, aufgefunden. Außer ihr befanden sich auf der Wiese noch: *Festuca valesiaca, Andropogon ischaemum, Chrysopogon gryllus, Adonis vernalis* u.a.

Obwohl JÁVORKA, ein gründlicher Kenner der pannonischen Flora und deren Vergangenheit, die Möglichkeit nahelegt, daß diese Pflanze adventiver Herkunft sei und in früheren Zeiten in die pannonische Region eingesiedelt wurde, muß gesagt werden, daß diese Ansicht mit einer gewissen Reserve angenommen werden sollte, zumindest hinsichtlich des Standortes, an dem wir sie vorgefunden haben. Es besteht ja im Grunde genommen kein wesentlicher Unterschied zwischen dieser Pflanze und anderer Arten, die nicht als adventive, sondern als regelrechte relikte

Bestandteile der pannonischen Flora angesehen werden: *Crambe tatarica, Adonis vernalis, Salvia nutans, Prunus tenella, Sternbergia colchicifloria* u.s.w. Zugunsten einer solchen Annahme spricht auch die Tatsache, daß die Pflanze bei uns inmitten einer natürlichen, durch menschliche Beeinflussung unberührten, steppenartigen Vegetation aufgefunden wurde. Außerdem muß noch hervorgehoben werden, daß sie auch auf dem Balkan, somit im zentralen Areal ihres Vorkommens, keineswegs oft vorkommt da sie Mitglied einer reliktartigen xenothermischen Flora ist. (Vgl. Abb. 1, 2).

*Ranunculus millefoliatus* VAHL.

Ihrem Charakter nach gehört diese Pflanze zu den submediterranischen Elementen der Flora und ist in den südlichen Gegenden Jugoslawiens ziemlich verbreitet. Von ihrem balkanischen Areal aus dringt sie nirgends in die Pannonische Tiefebene ein. In Serbien ist sie oft genug verzeichnet an mehreren Lokalitäten im Osten und Westen des Gebiets, in der Šumadija und am Kosovo (Flora Srbije Bd I — 1970). Die durch uns erfolgte Auffindung der Pflanze in der Sandlandschaft von Deliblato ist daher die erste und bisher einzige Feststellung ihres Vorkommens in der Pannonischen Tiefebene. In dem neuesten Werk von Soó über die ungarische Flora und Vegetation (1966) gibt es keine Angaben über ihr Vorkommen in dieser Flora. Die einzige Angabe über das Vorkommen dieser Art auf dem linken Donauufer befindet sich in der "Flora Rumäniens" (1953) und bezieht sich auf die Region von Craiova bei Calafat.

Die Stelle an der diese Pflanze vorgefunden wurde, befindet sich zwischen der Großen und Kleinen Tilva bei Flamunda. Die Flora der Umgebung war die typische halbgeschlossene Vegetation der Sandlandschaft von Deliblato, wo sich unter den pflanzengeografisch interessanten Arten die *Paeonia tenuifolia, Prunus tenella, Stipa capillata, Viola ajtajana, Viola neményiana, Pulsatilla australis, Iris pumilla, Astragalus dasyanthus* u.a. vorfanden, also Pflanzen, die heute in der Hauptsache zu den pontisch-mediterranischen Arten gehören, pontischen und endemischen Charakters sind und zum größten Teil Relikte aus einer vorhergegangenen Steppen-Periode darstellen.

Die Bedeutung der Auffindung dieser Pflanze liegt daher — abgesehen davon, daß sie eine außergewöhnliche floristische Neuheit betrifft — in erster Reihe in ihrem pflanzengeografisch-historischen Charakter.

*Doronicum orientale* HOFFM.
*Doronicum caucasicum M.B.*

Sie ist eine pontisch-balkanische Art, Einwohnerin der bewaldeten Zone. Als solche lebt sie in ihrer Heimat in lichten Wäldern und an den Abhängen in den Gebirgsgegenden des Kaukasus und auf dem Balkan (JÁVORKA, 1925). Derselbe Autor fand ihr Vorkommen an einigen Standorten in Transdanubien (Mecsek bis Szekszárd und Harsány-hegy), HORV. (Bezirk Pozsega). Wir fanden sie an den südlichen Abhängen der Fruška Gora, am Fuß des Crveni Čot, am Rande eines mesophilen Waldes. Trotz des mesophilen Charakters dieses Waldes gab es in ihm ziemlich viele balkanische und pontische Elemente: *Silene parviflora, Lychnis coronaria, Dianthus armeriastrum, Kitaibelia vitifolia, Ranunculus pedatus* u.s.w.

Es ist von Bedeutung, daß das Vorfinden dieser Pflanzenart teilweise zusammentrifft mit jenem einer anderen osteuropäisch-balkanischen Art, dem demselben

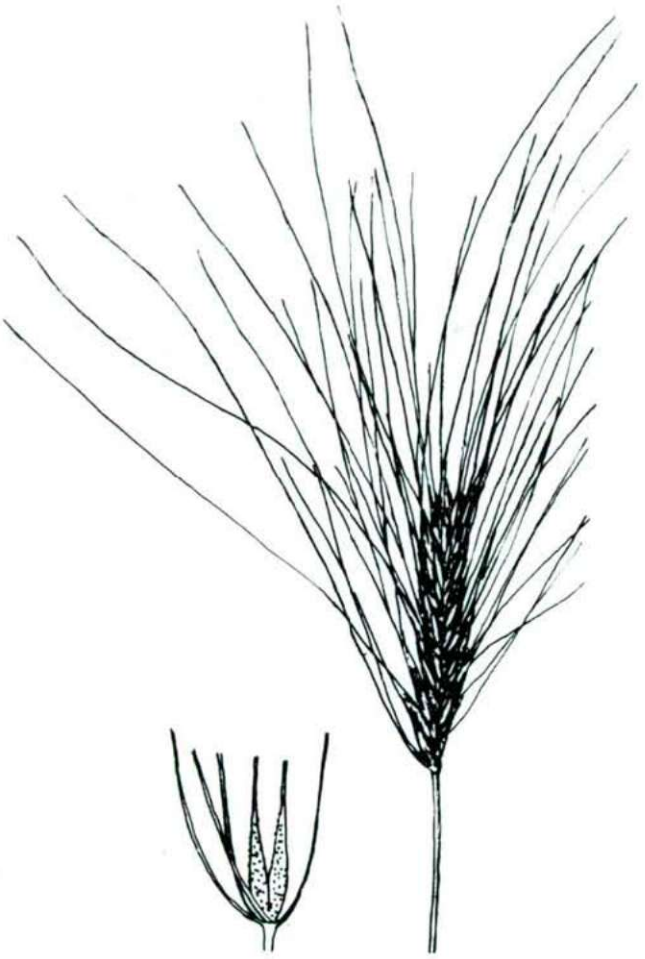


Abb. 1. *Taeniatherum asperum* NEVSKI (JÁVORKA1934)

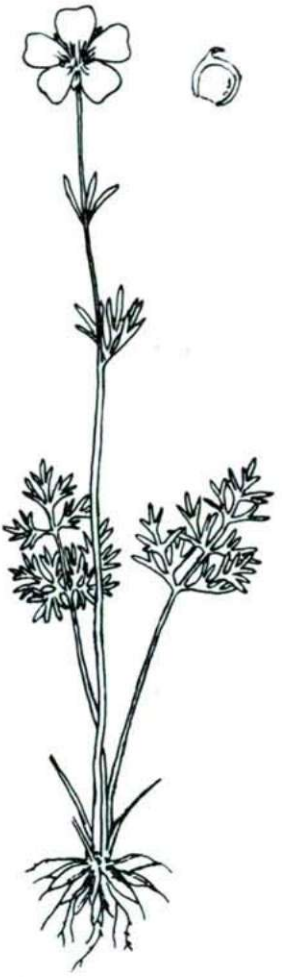


Abb. 2. *Ranunculus millefoliatus* VAHL. (Flora Srbije 1970)

Genus angehörenden *Doronicum hungaricum*. Diese letztere dringt jedoch weiter in die Pannonische Tiefebene vor und hat einen weniger ausgeprägten pontischen Charakter. Im Gegensatz zu ihm ist *Doronicum caucasicum* stärker auf das Vorkommen im Süden der Pannonischen Tiefebene beschränkt, seinen thermophilen Ansprüchen entsprechend.

Durch das Auffinden dieser Art wird der pontisch-mediterranische Charakterzug der Flora der Fruška Gora verstärkt. (Vgl. Abb. 3).

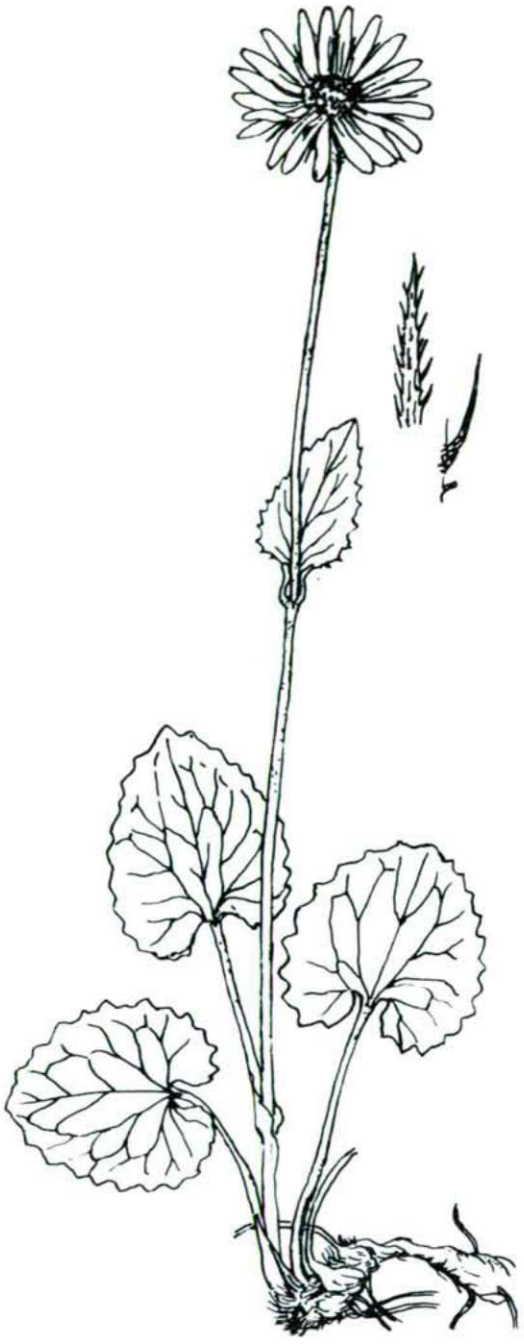


Abb. 3. *Doronicum orientale* HOFFM. (JÁVORKA 1934)

**Zusammenfassung**

Im Laufe unserer mehrjährigen Untersuchung der Flora der Vojvodina, am Südrand der Pannonischen Tiefebene, haben wir drei Pflanzen vorgefunden: *Taeniatherum asperum* NEVSKI, *Ranunculus millefoliatus* VAHL., und *Doronicum orientale* HOFFM. Alle drei Pflanzen sind in dieser Region neu und von gewisser systematischen und pflanzengeografischen Bedeutung. *Taeniatherum asperum* ist am Banstol, in dem östlichen Gebiet der Fruška Gora, ein Mitglied der natürlichen, von menschlicher Beeinflussung unberührten, Steppen-Vegetation und gehört daher zur relikten xerothermischen Flora. *Ranunculus millefoliatus* wurde in der Sandlandschaft von Deliblato zwischen der Großen und Kleinen Tilva bei Flamunde vermerkt. Die Vegetation der Umgebung, in welcher die Pflanze gefunden wurde, war eine typische Vegetation vom halbgeschlossenen Typ, mit Arten von pontisch-mediterranischen, pontischen und endemischen Charakter, deren größter Teil offensichtlich reliktartigen Charakters ist und aus einer vorangegangenen, wärmeren Steppenperiode stammt.

*Doronicum orientale* wies durch ihr Auffinden in der Fruška Gora, auf den höchsten Gipfeln des Gebirges, am Fuße des Crveni Čot (539 m). in einer Vegetation am Rande des mesophilen Eichen-Buchen-Waldes, von neuem darauf hin, daß es zur Gruppe der Gebirgspflanzen aus dem bewaldeten Bereich gehört, allenfalls aus den gelichteten Waldzonen. Es gab dort genug an Pflanzen vom balkanischen und pontischen Charakter. Durch das Auffinden dieser Art in der Fruška Gora wird der pontisch-mediterrane Charakterzug der Flora verstärkt.

**Literatur**

ČOLIĆ D. i BROZ V. (1969): Endemićne, retke i ugrožene biljne vrste Deliblatske peščare. Zbornik radova I. — Deliblatski pesak. Beograd.

Flora SR Srbije I—IV. (1970—1974): Srpska Akademija nauka i umetnosti. Beograd.

HAYEK A. (1927—1933): Prodromus florae peninsulae Balcanicae. — Berlin

HEUFFEL J. (1858): Enumeratio plantarum in Banatus Temesiensis — Vindobonae.

JÁVORKA S. (1925): Magyar Flóra (Ungarische Flora). — "Studium" Aufl. Budapest

JÁVORKA S. (1934): A Magyar Flóra képekben (Die ungarische Flora in Bildern). — "Studium" Aufl. Budapest.

KOVÁCS F. (1929): Óbecse határának virágos növényei (Die Blütenpflanzen der Dorfflur von Óbecse). Szeged.

OBRADOVIČ M. (1966): Biljnogeografska analiza flore Fruške gore — Matica srpska, Novi-Sad.

PRODÁN GY. (1916): Bács-Bodrog vármegye flórája. — Magyar botanikai lapok. Budapest.

ROCHEL A. (1828): Plantae Banatus rariores. — Pestini.

SIGUNOV A. (1970): Pregled flore Deliblatske peščare. Zbornik radova II. — Deliblatski pesak. Beograd.

SLAVNIČ Ž. (1953): Prilog flori našeg Podunavlja. — Glasnik biološke sekcije. 2. 13. Zagreb.

SOÓ R. (1966—1973): A Magyar Flóra és vegetáció rendszertani- növényföldrajzi kézikönyve II—V (Das systematische-pflanzengeographische Handbuch der ungarischen Flora und Vegetation II—V). Budapest.

ZORKÓCZY L. (1896): Újvidék és környékének flórája (Die Flora von Novi Sad und Umgebung). Újvidék.

Adresse des Autors:
Prof. Dr. MELANIJA OBRADOVIČ
Prirodna-matematicki fakultet
Institut za biologiju
21000 Novi Sad
Dr. Ilije Djuricica 2
SR Jugoslavija

Acta Biologica Szeged. *21* (1—4), pp. 69—82 (1975)

# EFFECT OF GROWTH-SUBSTANCES ON THE FREE AMINO-ACID CONTENT OF LENTIL SHOOTS IN CASE OF WATER DEFICIENCY

G. PÁLFI, ERZSÉBET KÖVES, and R. NEHÉZ

*Department of Plant Physiology, Attila József University Szeged, and Research Institute for Cereal Production, Szeged*

(Received October 20, 1974)

## Abstract

The free proline is not accumulated in an extremely high degree, as a result of a strong water deficiency, in the leaves of every plant species. From among 80 mono- and dicotyledonous herbaceous plant species belonging to fifteen families fifteen species of plants are storing proline but in a comparatively lower degree in case of water deficiency.

We have established which is — at time of a comparatively equal but strong water deficiency — the proline-concentration level in case of attainment of which we may speak of a "plant species of proline-accumulating type". This proline quantity is 10 mg/g dry matter. On the basis of that, the majority of the 80 plant species investigated, belonging mainly to the cultivated plants, may be regarded as proline-accumulating ones.

Studying the part of the free proline accumulated during water deficency, in the course of the developing or ceasing drought, it is not the same whether we are examinig a plant of proline-accumulating type, or not, because the ways of the free *aminr-acid* metabolism and enzyme systems of the non-proline-accumulating species differ from those of the proline-accumulating plants probably considerably.

We have established that in the course of the development of a strong water deficiency in the "proline-type" lentil shoots, the growth-substances applied (IAA, FAP, $GA_3$) exerted their influence on the water content of shoots and increased their free total amino-acid and proline content considerably.

The greatest effect was achieved by the 10 mg/l solution of IAA that increased the free total amino-acid content of lentil shoots 200 p.c., and their proline content 58 p.c., as compared with the water-treated control.

It can be concluded from the results of our investigations concerning the water balance that the IAA-treatment may be advantageous in regard of the drought-resistance of lentil shoots. This treatment provided, namely, the largest bound water content, as well.

In the course of our experiments, after IAA, the 20 mg/l FAP-treatment resulted in the largest increase in proline concentration; the 50 mg-l $GA_3$- treatment, however, increased proline-accumulation but in a lesser degree, and it had no considerable effect on the water balance, either.

In the course of our experiments, after IAA, the 20 mg-l FAP-treatment resulted in the largest increase in proline concentration; the 50 mg/l GA -treatment, however, increased proline-accumulation but in a lesser degree, and it had no considerable effect on the water balance, either.

We have demonstrated the physical, chemical, and physiological factors on the basis of which — at the proline-accumulating types — the high proline level is advantageous in regard of tolerating water deficiency.

## Introduction

We established in the course of our investigations lasting for several years on the subject of plants grown in the field and in culture-vessels (PÁLFI, 1968a, 1969, 1971; PÁLFI et al., 1973, 1974) that in the majority of mezophytes (herbaceous), in case of strong water deficiency, the total amino-acid and first of all proline content

of leaves significantly increased. A similar conclusion was drawn by BARNETT and NAYLOR (1966), BOKAREV and IVANOVA (1971), LEWITT (1972), VALLEE (1973), and PERDRIZET (1974).

According to the investigations of Stewart et al. (1966), that is a common phenomenon in the circles of the various plant families. KUDREV (1970), however, is taking the view that the level of proline accumulation is quite different according to plant species. In his opinion, if e.g. in bean proline achieves a medium level, a further synthesis comes to a stop, because of the end-product inhibition of enzymes.

Some have striven to clear up the path ways of proline production by applying inhibitors of growth, glycolysis, and respiration, respectively.

BRITIKOV and LINSKENS (1970) applied inhibitors of glycolysis (NaF and Na-azide) but they performed their experiments mainly on spinach, resp. maize and these plants, as we ascertained (PÁLFI, 1971; PÁLFI et al., 1973), don't store up a very large quantity of proline in the time of the greatest water deficiency, either.

According to STEWART et al. (1966), arsenate, iodine-acetate, and iodine-acetamide probably inhibit glycolysis, resp. the formation of alpha-ketoglutarate. Therefore, there cannot be produced any glutamic acid, the starting compound of proline synthesis.

In our earlier investigations (PÁLFI, 1968b), kinetine and 2.4—D did not prevent water-deficient plants from accumulating proline resp. a large amount of free amino acid, and proline synthesis was not brought to an end by maleic hydrazide and chloramphenicol, either. Only 2.4-dinitrophenol was effective in this respect — by uncoupling the oxidative phosphorilation. And as proline synthesis demands ATP, its accumulation did not take place.

In our present experiment we have first of all endeavoured to clarify if every species of herbaceous mesophytes is storing proline in an extremely large measure in its leaves, in case of strong water deficiency achieving 2—4 per cent of dry matter.

We are investigating, as well, which is the proline concentration after achieving or exceeding of which we may speak of an extraordinary proline accumulation; further on, if a high-level proline accumulation is advantageous to plants in regard of tolerating water deficiency.

Finally, we have studied whether some growth-regulating substances: indole-3--acetic acid, furfurylaminopurine, gibberellic acid increase or decrease the total amino-acid or proline synthesis and accumulation during water deficiency. We are appreciating, too, the influence of growth-substances on water balance, on the quantity of dry matter and soluble total protein.

The effect of growth-stimulating substances on the water balance of plants was investigated recently (TRUNOVA, 1968; MIZRAHI et al., 1970; DERBYSHIRE, 1971; ITAI and VAADIA, 1971; TUCKER and MANSFIELD, 1971; LOESCHER and NEVINS, 1973).

BADANOVA and LEVINA (1970) established in barley that drought-resistance was increased somewhat by CCC, and decreased by $GA_3$.

ZADANTSEV and PIKUS (1973) found so that in case of wheat CCC was promoting to tolerate water deficiency and high temperature. The changes in free amino acids were, however, outside the scope of their investigations.

## Materials and Methods

For clearing up the type of amino-acid metabolism during drought respectively for classifying the plants, we have mostly collected the shoots or leaves of plants grown in field. The shoots of entil *(Lens culinaris)* were grown in culture-vessels by soil culture.

The solutions of indolacetic acid (IAA), kinetine (furfurilaminopurin—FAP), and gibberellic acid ($GA_3$) were prepared from the chemicals of the firm E. Merck, Darmstadt.

For analysing amino acids, we have started from 200 mg leaves dried up till getting steady weight at 60° C and pulverized. The qualitative analysis of amino acids was carried out in a part of experiments by means of ascending paper chromatography. We have worked by developing with retarded cooling, the solvent being butanol-acetic acid-water (3:1:1), and a tproline detections phenol-water (4:1).

We had already published the method of measuring free proline and total amino acid by elu-ation (PÁLFI, 1968a, 1971).

At measuring total amino acid, we have eluted with methanol, and at proline determinations we have done that with water-saturated phenol. At total amino-acid determinations, the calibration curve was constructed from the measurements of series of different concentration of the so-called "universal standard mixture" composed by us from sixteen amino acids. The composition of stan-dard, in qualitative as well as quantitative respects, was similar to the pattern of plant extracts.

The soluble total protein was determined according to LOWRY et al. (1951), and tris-buffer was used for preparing extracts (pH 7.5).

At the automatic amino-acid analyser "BIOCAL BC 200" buffers of 3.25, 4.25, and 5.28 pH-value were applied.

The mean error in the average results of the quantitative analyses carried out in 3 to 15 repe-titions, that is their standard deviation was less than $\pm 5$ to 7 per cent.

## Experimental results

### 1) "Proline" and "non-proline" types of the amino-acid accumulation in the course of water loss.

In the course of our experiments we have taken into consideration ourearlier estab-lishment (PÁLFI, 1973, PÁLFI et al. 1974) that in the time of the so-called "live-wilting", i.e., the gradual water loss of the detached shoots and leaves during being lighted, there are taking place the same biochemical processes as in case of intact plants. The difference is mainly that for the formation of a strong water deficiency in the native plants grown in the field a four to six weeks long time without precipitation resp. irrigation being necessary. The same high-level water deficiency in detached leaves is attainable already in two to five days. During that time, the total amino-acid content and proline-level are increasing to the highest degree, the same as in the leaves of native plants.

With the method, elaborated by us, of "live-wilting" the leaves and shoots detached, we have investigated the free amino-acid, resp. proline concentration of 80 plant species of 15 families, in case of an optimum water supply and in that of a developing, strong water deficiency.

"Live-wilting" took place for four to five days, under permanent lighting (5000 Lux), while we achieved a 40 to 45 per cent water deficiency in the (green) plant parts detached (in the percentage of the fresh matter in the varieties irrigated). From the results obtained, we are giving some data in Table 1.

It turns out from the data of Table 1 that, at any mono- and dicotyledons, the total amino-acid resp. proline content is significantly increased. While anyway the quantity of total amino acid in all the species has increased to be three-six-times as

*Table 1.* Free proline and total amino-acid content of detached leaves and shoots of mono- and dicotyledonous soft-stalk plants, as a result of a water loss for 4 to 5 daysat 25° C, besides 60 per cent relative vapour content and illuminated permanently with 5000 Lux, in the prebloom period of development. The proline content of the leaves of plants grown under optimum water conditions was below 0.4 mg/g dry matter, and their total amino-acid content below 20.0 mg. The species were arranged in families and separated with line. (The average deviation of the analyses carried out in three repetitions was below ±7 per cent.)

| Plants | Proline | Total amino acid | Plants | Proline | Total amino acid |
|---|---|---|---|---|---|
| | mg/g dry matter | | | mg/g dry matter | |
| *Sinapis alba* | 24.8 | 65.4 | *Helianthus annuus* | 21.4 | 47.3 |
| *Raphanus sativus* | 26.4 | 63.2 | *Artemisia vulgaris* | 23.7 | 52.8 |
| *Brassica napus* | 25.9 | 57.5 | *Matricaria chamomilla* | 22.1 | 56.8 |
| *Brassica oleracea* | 43.7 | 110.2 | *Lactuca sativa* | 3.0 | 49.6 |
| *Solanum tuberosum* | 29.6 | 82.5 | *Taraxacum offici.* | 4.2 | 45.7 |
| *Capsicum annuum* | 35.2 | 53.7 | *Pisum sativum* | 33.1 | 106.3 |
| *Nicotiana tabacum* | 28.5 | 75.3 | *Lens culinaris* | 22.1 | 70.4 |
| *Sol. lycopersicum* | 22.3 | 63.2 | *Medicago sativa* | 20.3 | 104.6 |
| *Hyoscyamus niger* | 26.5 | 68.4 | *Trifolium repens* | 21.4 | 89.6 |
| *Sol. laciniatum* | 32.6 | 72.1 | *Phaseolus vulg.* | 8.6 | 53.4 |
| *Triticum aestivum* | 25.4 | 82.4 | *Chenopodium album* | 4.7 | 70.1 |
| *Hordeum vulgare* | 18.6 | 59.9 | *Spinacia oleracea* | 4.2 | 72.5 |
| *Secale cereale* | 17.9 | 64.5 | *Beta vulgaris* | 3.8 | 67.5 |
| *Avena sativa* | 21.3 | 54.6 | *Rumex scutatus* | 3.3 | 54.7 |
| *Sorghum vulgare* | 20.4 | 58.2 | *Anethum graveol.* | 18.2 | 56.6 |
| *Cynodon dactylon* | 25.7 | 60.1 | *Rubus caesius* | 21.8 | 54.0 |
| *Festuca pratensis* | 22.8 | 63.7 | *Allium cepa* | 4.2 | 47.6 |
| *Bromus arvensis* | 26.4 | 52.8 | *Allium sativum* | 3.6 | 52.9 |
| *Poa pratensis* | 23.6 | 54.5 | *Cucurbita pepo* | 3.4 | 53.2 |
| *Lolium perenne* | 31.4 | 63.6 | *Cucimis sativus* | 3.0 | 44.5 |
| *Lolium aristatum* | 28.5 | 71.0 | *Cucumis melo* | 2.9 | 47.6 |
| *Zea mays* | 5.1 | 53.6 | *Colocynthis citrullus* | 3.5 | 56.2 |
| *Vitis vinifera* | 7.9 | 45.6 | *Papaver somniferum* | 4.0 | 41.7 |

the (non-water-deficient) control, the proline concentration has given quite striking differences in the various species. The increase in proline content is, therefore, changing from sixfold *(Cucumis melo)* till 135-fold one *(Capsicum annuum)*. It follows from the data that it is to be decided, which level is to be regarded as an extraordinary increase in proline, respectively as a proline-type amino-acid metabolism in the course of water deficiency.

On the basis of our several experimental data, we consider as a proline-accumulating type during water-deficiency the herbaceous mesophytous plant species

the young but full-developed green leaves of which native plant, or the shoots detached and leaves isolated of which, in the course of their "live-wilting", resp. during losing gradually 40 to 50 per cent of their water content at being illuminated, accumulate at least 10 mg free proline, as related to 1 g dry matter. (The determination is to be performed in the phase of budding, resp. blooming, at a temperature and vapour content that correspond to the water loss becoming gradually ısronger, resp. to the „live wilting" in case of any plant species.)

It turns out from Table 1, as well, that not every plant species may be regarded as a proline-accumulating type, in case of water-stress, as we have obtained in 15 plant species of the Table a proline quantity that is considerably below 10 mg. We have, therefore, to abstain from the fact of a general, extraordinary proline accumulation as a result of a strong water deficiency in plants.

It is anyhow to be noted that in the Table 34 plant species of proline-accumulating type are not published from among the species investigated so far. Taking all this into consideration, we may establish that, on the basis of our investigations, the majority of plant species belong to the proline accumulating type.

In the course of our experiments, we laid the main stress upon screening the proline-accumulating species surely. That is not an easy task because if we hadn't achieved a 10-mg level at some species in case of a strong water deficiency, either, then we often had to repeat the experiment, under the conditions of a water loss of quite different degree and rate. In the course of our careful investigations carried on for several years, we fell nevertheless into error not only on one occasion. At any rate, in Table I, we are publishing the highest values of proline content from among those of non-proline-type species measure during the repeated investigations.

From our results obtained, we may already draw the conclusion that before beginning to investigate the free amino-acid spectrums and protein synthesis developing as a result of water deficiency, we have to establish the type of accumulation at every species. The experiments may only be continued after taking into consideration these data because the reaction ways and regulative mechanisms of the proline accumulation of extraordinary size are differing from those in plants of non-proline type. That was established by TYANKOVA (1969), as well.

In the course of our experiments, we established undoubtedly and several times that lentil (and 70 to 80 per cent of the species investigated of the Leguminosae family) belong surely to a type accumulating proline in case of a strong water deficiency. This is the reason of choosing lentil for the aim of our present research work.

### 2) The effect of growth-substances upon accumulating free amino acids in the course of water loss

From the isolated lentil shoots we weighed out equal quantities and, after eluting them carefully, we evaporated the water adhering to the surface. Then we had the water, resp. growth-substances weighed in an exact quantity into the beakers, absorbed through the stalk and at being illuminated, for 24 hours (Fig. 1). In the meantime, in order to avoid infections, we weighed and changed the fluids of tumblers in six hours. At being placed into the solution in beakers, the shoots and leaves were somewhat wilted. The full turgor, resp. normal water supply was regained by any sample already in two to three hours after being steeped in the fluids.

After treating them for a day, the weight of lentil shoots was established in a water-saturated state. Then followed the "live-wilting" for 48 hours, by illuminating

Fig. 1. Treatment of 4-week detached lentil shoots (*Lens culinaris* L.), grown in culture, with the solutions of growth-regulating substances, for 24 hours, illuminated with 5000 Lux. Varieties: "AB" = tap water; "C" = IAA 10 mg/l; "D" = FAP, 20 mg/l; "E" = $GA_3$, 50 mg/l.

the samples (5000 Lux) and engendering a gradual water loss, during which the 60 to 70 per cent relative vapour content of air was provided by means of a foil-cover.

After "live-wilting" for two days, we have weighed the samples (withering weight), then we took samples for determining the soluble total protein. Then, fixing the single varieties, we weighed their dry matter, as well. The data are given in Table 2.

It turned out from Table 2 that the lentil shoot of 100.0 g fresh weight absorbed a considerable fluid quantity during the 24 hours. Most water was absorbed by the sample placed into the FAP solution, and least of that by the sample treatment by $GA_3$. But in the water quantities absorbed there wasn't found any considerable difference. The water quantity stored in the plant is proving that a water saturation was produced and the fixation of water of different quantities was made possible

*Table 2.* The fresh weight of the lentil shoots pre-raised under identical conditions and detached (100.0 g), the amount of water absorbed during 24 hours and the weight and dry matter after being wilted in light during the following 48 hours, as a result of water, IAA, FAP, and $GA_3$. The starting amount of solutions is 150.0 ml. The average deviation is below ±5 per cent.

| Treatments of lentil shoots (The total weight of one sample is 100.0 g fresh matter) | Quantity of solution absorbed during treatment ml | Increase in weight of 100 g shoot after treatment g | Weight of shoots afrer the live-wilting for 48 hours g | Final (dry) weight of 100 g shoot g |
|---|---|---|---|---|
| Shoots fixed immediately after detachment (fresh, control) | — | — | — | 21.8 |
| Shoots fixed after a water treatment for 24 ours following detachment (control) | 76.0 | 12.1 | — | 21.3 |
| Held in water for 24 hours and "live-wilted" for two days | 76.8 | 12.4 | 35.6 | 20.2 |
| Held in the solution of IAA 10 mg/l for 24 hours and „live-wilted for two" days | 78.2 | 16.0 | 37.1 | 21.1 |
| Held in the solution of FAP 20 mg/l for 24 hours and "live-wilted" for two days | 80.3 | 15.8 | 34.0 | 20.6 |
| Held in the solution of $GA_3$ 50 mg/l for 24 hours and "live-wilted" for two days | 75.5 | 14.5 | 35.9 | 21.0 |

by the growth-substances applied. The water content of lentil shoots was most increasing as a result of being treated by the IAA solution (16.0). The bending of shoots (Fig. 1) is, therefore, not a consequence of water deficiency, resp. of wilting. It was also established that the bend of lentil shoots intensifies owing to the concentration of IAA solutions from 10 to 50 mg/l.

Fig. 2. Post-treatment wilting of lentil shoots detached, for 48 hours, under 5000 Lux illumination, below foil cover.
Varieties are the same as in Fig. 1, except for the watered control that was already fixed.

The water content of shoots was increased by being treated with FAP and $GA_3$ solutions, as well, as related to the control shoots treated with tap water.

It can be established from Table 2, as well, that the absorption treatment with IAA solution promoted not only the absorption of the largest water quantity but, after "live-wilting" for two days, the strongly fixed water content, too, remained the largest in case of this variety (37.1), although the difference between varieties is not considerable. In the dry matter of shoots we have not obtained any important differences, either, but in case of the growth-substances the final dry-weight is generally smaller than that of controls, that is to say, than that of the freshly fixed one and the variety administered with water for 24 hours.

From among the results of the qualitative amino-acid analysis, we are demonstrating an ascending one-dimension paper chromatogram (Fig. 3).

Taking into consideration that in the chromatogram seen in Fig. 3 we let run a standard mixture of the amino acid of known composition and concentration (stripe A), as well, we can draw approximative quantitative conclusions, too. We can establish that the total amino-acid content, as a result of "live-wilting" for two days, in all the four varieties, has increased to at least a double one (stripe CDEF), as compared with the control (stripe B). Most total amino acid, resp. proline, accumulated during being treated by IAA-solution (stripe F).

From Fig. 3 it can be established, too, that — compared with the samples fixed immediately after being cut (stripe B, fixed freshly) — about 15 to 20 per cent amino-acid increase was realized during the water imbibation lasting for 24 hours (stripe G).

In the course of this water imbibation of the lentil shoots detached, taking place in a bunch for 24 hours, particularly the quantity of leucine, phenylalanine, valine and methionine, pipecolic acid, asparagine and cysteine increased.

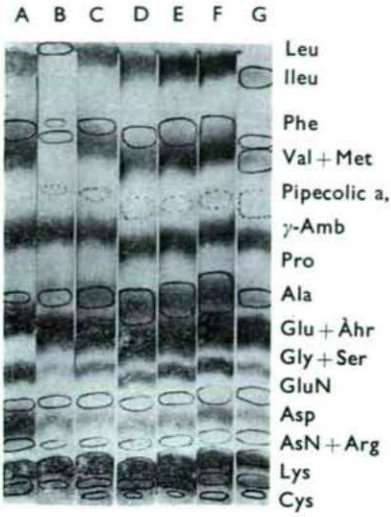



Fig. 3. Change in the free amino-acid composition in the lentil shoots detached, as a result of a 24-hour treatment and a "live-wilted" in light, for 48 hours. Control: shoots fixed immediately after being detached and the variety held in water. Ninhydrinic colour reaction, fixed with copper nitrate.[1]
"A" Comparing standard, its total amino-acid content being 60 μg; "B" = fixed immediately after being detached (fresh control); "C" = heldin water and "live-wilted" for two days (with gradual water loss); "D" = treated by FAP-solution and "live-wilted" for two days; "E" = treated by $GA_3$—solution and "live wilted" for two days; "F" = treated by IAA-solution and "live-wilted" for two days; "G" = fixed after being steeped in water for 24 hours (control).

It was determined the quantity of the free total amino acid and proline, as well as that of the soluble total protein, too. Results are published in Table 3.

From Table 3 it is to be established that in the course of the water-losing "live-wilting" lasting for two days the proline content accumulated to the highest degree as compared with the control supplied with water to the optimum degree. The highest proline level was however induced, as established at Fig. 3, as well, by the administration with IAA-solution. It is proved by the data, too, that the lentil plant belongs, in case of drought, really to the proline-accumulating type as the free proline content of all the four kinds of the "live-wilting" varieties (with water deficiency) has exceeded the 10 mg/g dry-matter level. It is to be noted, as well, that in case of lentils precultivated under optimum conditions in the field, in the course of losing water at "live-wilting", a much higher proline content can be achieved than the quantities included in Table 3.

In regard of the total free amino acid, as well, the highest value was given by the treatment by IAA-solution, as it has increased to 300 per cent of the fresh control. Apart from that, the total amino-acid content of every water-losing variety surpasses the double amount of the control considerably. The proline, resp. total amino-acid content was increased, to a greater or lesser degree, not only by being treated by IAA-but also by FAP and $GA_3$-solutions, as compared with the water-treated control that was wilted for two days.

*Table 3.* Free proline, total amino-acid and soluble total protein content of the lentil shoots pre-cultivated under identical conditions and detached (100.0 g fresh matter), as a result of stepping treatment in an agent for 24 hours and water-losing "live-wilted" at light for 48 hours. (The average deviation is below ±7 per cent. The control is a water-treated variety.)

| Treatment of the lentil shoots detached: 100.0 g fresh matter | Free proline | Total amino acid including proline | Soluble total protein: mg/g living matter |
|---|---|---|---|
| | mg/g dry matter | | |
| Shoots fixed immediately after detachment fresh, control | 0.32 | 14.9 | 18.8 |
| Fixed after water treatment for 24 hours following detachment (control) | 0.31 | 17.3 | 18.5 |
| Held in water for 24 hours and "live-wilted" for two days | 11.6 | 34.8 | 18.4 |
| Held in the solution of IAA 10 mg/l for 24 hours and "live-wilted" for two days | 18.4 | 49.6 | 18.0 |
| Held in the solution of FAP 20 mg/l for 24 hours and "live-wilted" for two days | 15.9 | 40.9 | 18.4 |
| Held in the solution of $GA_3$ 50 mg/l for 24 hours and "live-wilted" for two days | 15.3 | 44.1 | 18.6 |

Although the highest value in regard of the soluble total protein content was given by the sample fixed immediately after being cut, there is no significant difference between the single samples as the differences fall under the error limit of average results.

We have checked our data of amino-acid analysis by means of an automatic amino-acid analyser of type "BIOCAL BC 200", as well. Four curves of the results are shown in Fig. 4.

In the course of this analysis, too, the free proline content of the sample treated by IAA-solution increased to the highest degree as compared with the contro- that was held in water. We established, too, that the proline content of lentil shoots was increased by the FAP- and $GA_3$-solution treatment, as well, and that in the figure from among all the amino acids the quantitative changes in proline are the most significant.

3) The significance of the high proline accumulation takingplace- in the course of losing water in regard of tolerating water deficiency

Further on, we have wanted to establish the advantages of a higher proline accumulation in tolerating water deficiency in case of the single plant species.

At the proline-type plants we have established that a high proline concentration is advantageous and we are supporting that as follows:

1) The hygroscopic nature of proline and its water-fixing capacity is the highest among all the protein-forming amino acids. Its water-solubility is standing in the

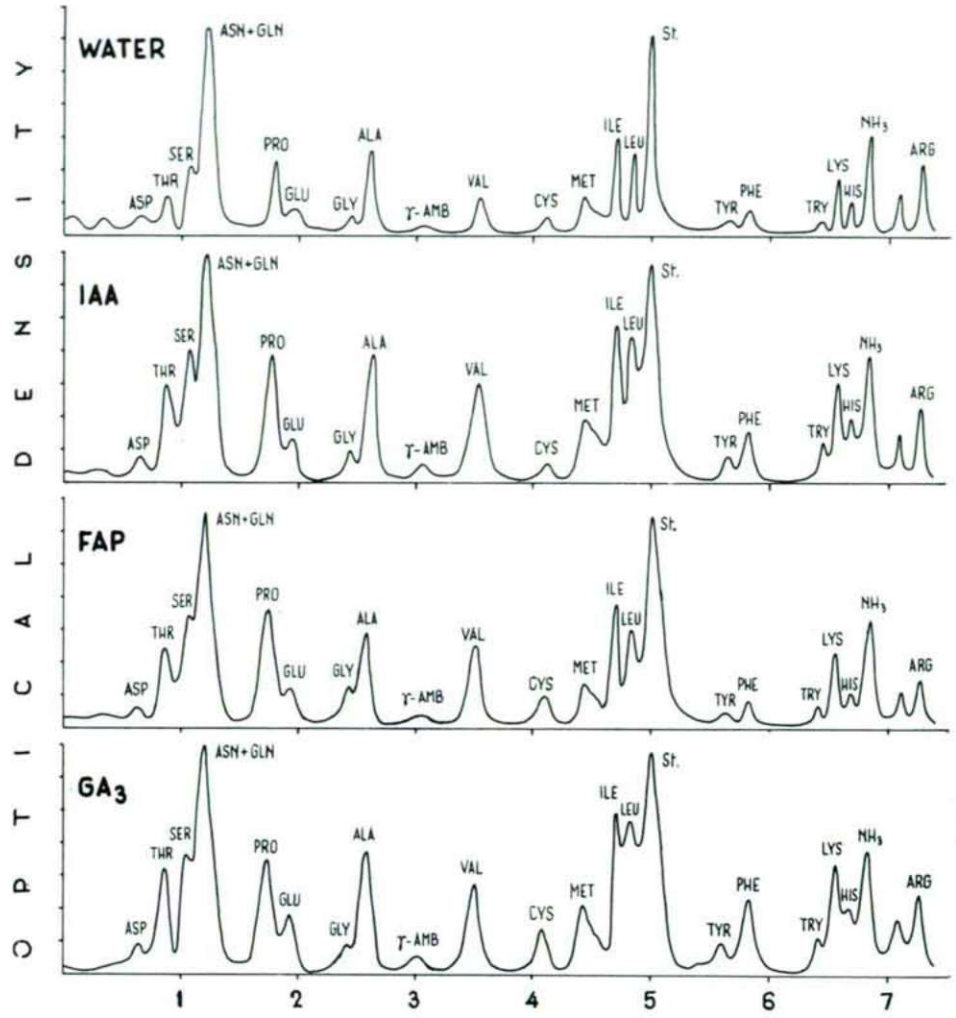



Fig. 4. Free amino-acid content of water-deficient lentil shoots, "live-wilted" for 48 hours after being treated, measured with an automatic amino-acid analyser of "BIOCAL BC 200"-type. Curves downwards: Water-, IAA-, FAP-, and $GA_3$-treated samples. (Average deviation being below $\pm 5$ per cent.)

first place, too: at syntheses and transaminations, the most frequently involved glutamic acid is soluble 192-times, and aspartic acid 300-times more poorly in water than proline does. It may therefore be in the tissues of plants in a dissolved, active state, even in the more and more decreasing water (physical advantage).

2) During hydrolysis the free amino acids with 6 n HCl, for 24 hours, at 110° C, under pressure — in the presence of $KNO_3$ as oxidizer — every protein-forming

amino acid was decomposed, except for proline. The proline-stability is extremely high (chemical advantage).

3) Proline, during its being formed from glutamic acid, is storing reducing energy coming from photosynthesis and that gets released after the water deficiency being ceased and proline reverting to glutamic acid again. Owing to this redox property, proline has a respiration-influencing role, as well.

4) The high concentration of free proline in the tissue — as compared with the other amino acids — is favourable to growth because it is less toxic, as we have proved that with germinating experiments carried out under sterile conditions and with an oat coleoptil test (physiological advantages).

On the basis of our results we can establish that a high-degree proline accumulation is favourable to plants in the period of tolerating drought. Therefore, in case of proline-type species, we can predict the beginning of water deficiency by demonstrating the proline quantity accumulated during the drought (PÁLFI and JUHÁSZ, 1971). The method can be applied to selecting the drought-resistent plant species, as well (PÁLFI et al., 1973, SINGH et al., 1972). That is confirmed by the experimental results of *Waldren* and *Teare* (1974), too.

## Evaluation of results

We have cleared in the first part of our experiments if each of the herbaceous mesophytous plant species, mono- and dicotyledons, accumulates proline to a great extent in case of water deficiency. On the basis of the extent of proline accumulation, the plants were classified into two types. If the proline concentration that is developing in the leaves of the plant in light gradually in case of a strong water deficiency reaches or surpasses the 10 mg/g dry matter quantity, the species is, in our opinion, a proline-accumulating one and the emerging picture of amino acid is of "proline type".

From among the 80 species investigated, only 15 did not prove to be proline accumulating-ones. The amino-acid picture of the majority of plants investigated is, therefore, of "proline type". The proline content of bean shoots often approximated the 10 mg/g quantity in case of water deficiency but it did not surpass it. We consider it, therefore, as an intermediate type.

It was established also by KUDREV (1970) that, in case of bean, the proline accumulated exerts an effect on the enzymes taking part in the synthesis by means of end-product inhibition. Its storing comes therefore to a stop at a certain level of concentration.

We cannot consider as final, therefore, the establishment of STEWART et al. (1966) that a considerable accumulation of proline in case of water deficiency is a common phenomenon at plants. It is true that the authors drew that inference on the basis of their results obtained only at eight plant species investigated. STEWART (1971, 1972) examined also the biochemical ways of transformation of the proline of exogenous origin in bean plants, in the dark. According to us, the behaviour of bean is not characteristic of proline-type plants in this regard, either.

In case of lentils, on the other hand, we may regard as advantageous to hold amino acid, resp. proline in store as this species belongs to the proline-accumulating type.

On the basis of water balance, we may consider as proved that the treatment with IAA, respectively the increased amino-acid content, as well, can be regarded as advantageous for tolerating drought. If we investigate, namely, the data obtained in case of lentils concerning water balance, then it turns out that the difference at the various treatments is not considerable as regards water uptake and water loss but the water content bound in the shoots has grown the most as a result of IAA-treatment. That variety contained most water after withering two days long, as well. It follows from that the bend experienced at lentil shoots is not a consequence of the decrease in turgor but it is epinasty.

The cold-resistance of plants was investigated by TRUNOVA (1968). According to her, as well, the water flow out of the cells is reduced by IAA-treatment and with that the water content of cells is increased. In respect of cold-resistance, this phenomenon is anyway not advantageous because if water remains inside the cell and freezes there then the plant perishes.

IAA-experiments were carried out by KUDREV and TYANKOVA (1966), as well. They were, however, examining if after the water deficiency that the plants had survived, the quick rehydration and the normalization of the amino-acid metabolism were advanced by IAA.

KUDREV and TYANKOVA experimented with full-grown wheat of proline-accumulating type and they applied 100 mg/l IAA-concentration. In their opinion, IAA undeniably promoted the restoration of water balance and a quick decrease in the high concentration of free amino-acid and proline, respectively.

Bin and STANLEY (1972) examined the effect of IAA-solution on the water balance in the stalk-segments of peas. According to them, the water flow was accelerated by the 10—30 μMol concentration of IAA in both directions, that is to say, in the tissues both inwards and outwards (and even inside of the tissues, as well). In the authors opinion, the IAA-solution exerts its effect on the permeability of membrane systems.

LIVNE and GRAZIANI (1972) established concerning kinetine (FAP) that it filled an important part in regulating the water-permeability of leaf-tissues. In the course of our experiments, the treatment with FAP-solution gave the second largest increase in proline concentration, after IAA, as compared with the control.

BADANOVA and LEVINA (1970) established that after spraying barley plants with 0.01 per cent $GA_3$-solution the thermo- and drought-tolerance of plants decreased. In our experiments proline-accumulation was promoted by $GA_3$ but in a lesser degree.

At any rate, from our results the conclusion may be drawn that from among all the hormones the IAA-treatment was the most effective for raising both the level of the total amino-acid and proline increase, and that of the water balance of shoots. It is probable that this effect of the hormones is indirect and its mechanism is complicated that demands further studies.

## References

BADANOVA, K. A., LEVINA, V. V. (1970): O vliinaii gibberellina i retardanta CCC na zasuhoustoičivost iačmen'a. — Fiziol. Rasteniy. *17*, 568—573.

BARNETT, N. M., NAYLOR, A. W. (1966): Amino acid and protein metabolism in Bermuda grass during water stress. — Plant Physiol. *41*, 1222—1230.

BIN, K. G., STANLEY, B. G. (1971): Rapid change in water flux induced by auxins. — Proc. Nat. Acad. Sci. *68*, 1730—1733.

BOKAREV, K. S., IVANOVA, R. P. (1971): Deistvie nekotoryh proizvodnyh i analogov holina i betaina na soderzanie svobodnyh aminokislot v listiah dvuh vidov kartofel'a, otličaiuschihsia po ustoičivosti k zamorozku. — Fiziol. Rastenii. *18*, 365—368.

BRITIKOV, E. A., LINSKENS, H. F. (1970): Vliyanie prolina na pogloshčihhenie kisloroda tkanyami rasteniy. — Fiziol. Rasteniy. *17*, 645—654.

DARBYSHIRE, B. (1971): The effect of water stress on indolacetic acid oxidase in pea plants. — Plant Physiol. *47*, 65—67.

ITAI, C., VAADIA, Y. (1971): Cytokinin activity in water stressed shoots. — Plant Physiol. 47, 87—90.

KÜDREV, T. G. (1970): on the problem of the formation of proline under the effect of drought. — Dokl. Acad. Selhoz. Nauk v Bulg. *3*, 261—264.

KÜDREV, T. G., TYANKOVA, L. (1966): Vliyanie IUK i 2,4-D na soderzanie svobodnüh i svyazannüh aminokislot psenicü posle vozdeystviya kratkovremennoy zasuhi. — Fiziol. Rasteniy. *13*, 988—995.

LEWITT, J. (1972): Responses of plants to environmental stresses. — New York and London.

LIVNE, A., GRAZIANI, Y. (1972): A rapid effect of kinetin on rehydration of tobacco leaf tissue. — Plant Physiol. 49, 124—126.

LOESCHER, W. H., NEVINS, D. J. (1973): Turgor-dependent changes in Avena coleoptile cell wall compositions. — Plant Physiol. 52, 248—251.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L., RANDALL, R. J. (1951): Protein measurement with the folin phenol reagent. — J. Biol. Chem. 193, 265—275.

MIZRAHI, Y., BLUMENFELD, A., RICHMOND, A. E. (1970): Abscisic acid and transpiration in leaves in relation to osmotic root stress. — Plant Physiol. *46*, 1169—1171.

PÁLFI, G. (1968a): Changes in the amino acid content of detached wilting leaves of Solanum laciniatum Ait. in the light and in the dark. — Acta Agron. Acad. Sci. Hung. *17*, 381—388.

PÁLFI, G. (1968b): Die Wirkung von Kinetin, 2,4-DNP und Antimetaboliten auf die Veränderungen im Aminosäurengehalt welkender Pflanzenblätter. — Planta (Berl.) *78*, 196—199.

PÁLFI, G. (1969): Das Prolin, die dem Wassermangel der Pflanzen anzeigende Aminosäure. — Acta Biol. Szeged. *15*, 65—69.

PÁLFI, G. (1971): Multiplication of the essential amino acids during the live-wilting of leaves. — Acta Biol. Szeged. *17*, 89—103.

PÁLFI, G., BITÓ, M., PÁLFI, Zs., (1973): Svobodnüy prolin i vodnüy deficit rastitelnüh tkenay. — Fiziol. Rasteniy. *20*, 233—237.

PÁLFI, G., JUHÁSZ, J. (1971): The theoretical basis and practical application of a new method of selection for determining water deficiency in plants. — Plant and Soil. *34*, 503—507.

PÁLFI, G., ERZSÉBET KÖVES, NEHÉZ R. (1974): Main types of amino acid regulation in cultivars with deficient water supply, and their practical application in agriculture. — Növénytermelés. *23*, 219—228.

PERDRIZET, E. (1974): Vliyanie nedostatochni klorofilla na metabolizm prolina u vüsshih rasteniy. — Fiziol. Rasteniy. *21*, 61—68.

SINGH, T. N., ASPINALL, D., PALEG, L. G. (1972): Proline accumulation and varietal adaptability to drought in barley: a potential metabolic measure of drought resistance. — Nature New Biology. *236*, 188—189.

STEWART, C. R. (1971): Effect of wilting on carbohydrates during incubation of excised bean leaves in the dark. — Plant Physiol. *48*, 792—794.

STEWART, C. R. (1972): Effect of proline and carbohydrates on the metabolism of exogenous proline by excised bean leaves in the dark. — Plant Physiol. *50*, 551—555.

STEWART, C. R., MORRIS, C. J., THOMPSON, J. F. (1966): Changes in amino acid content of excised leaves during incubation. — Plant Physiol. *41*, 1585—1590.

TRUNOVA, T. I,(1968): Vliyanie indoluksusnoy kislotü na morozostoykost ozimüh zlakov.—Fiziol. Rasteniy. *15*, 773—777.

TUCKER, D. J., MANSFIELD, T. A. (1971): A simple bioassay for detecting "antitranspirant" activity of naturally occurring compounds such as abscisic acid. — Planta (Berl.) *98*, 157—163.

TYANKOVA, L. (1969): Changes in the content of sugars and the free proline in saccharose-enriched and non-enriched chlorophyllic and non-chlorophyllic mutant tomato and pepper plants under wilting. — Dokl. Acad. Selhoz. Nauk v Bulg. *2*, 221—224.

VALLEE, J. K. (1973): Svobodnüy i svyazannüy prolin u tabaka Nicotiana tabacum var. Xanthi n. c. v svyazi s razvitiem i temperaturnümi usloviyami. — Fiziol. Rasteniy. *20*, 1109—1116.

WALDREN, R. P., TEARE, I. D. (1974): Free proline accumulation in drought stressed plants under laboratory conditions. — Plant and Soil. *40*, 689—692.
ZADANCEV, A. I., PIKUS, G. R. (1973): Hlorholinchlorid v rastenievodstve. — Kolos. 360.

Address of the authors:
Dr. G. PÁLFI
Dr. ERZSÉBET KÖVES
Department of Plant Physiology,
A. J. University,
H—6701 Szeged, P. O. Box 428,
Dr. R. NEHÉZ
Research Institute
for Cereal Production,
H—6726 Szeged, Hungary

Acta Biologica Szeged. *21* (1—4), pp. 83—87 (1975)

# DEPENDENCE OF POTASSIUM UPTAKE OF RICE AND WHEAT SEEDLINGS ON TEMPERATURE, PRESENCE OF CALCIUMION AND ROOT LENGTH

F. ZSOLDOS

*Department of Plant Physiology, Attila József University, Szeged*

(Received September 20, 1974)

## Abstract

A study was made of the effect of temperature on the K-ion uptakes of rice and spring wheat seedlings. The uptake studies were carried out in $10^{-3}$ M K($^{68}$Rb)Cl solution in the presence and absence of Ca-ion and at different root lengths (ages). The experimental data indicated the following: the lower the temperature of the absorption solution, the higher the K-ion uptake anomaly of excised rice roots; no departure from the regular K-ion uptake was revealed by using wheat seedlings (roots) at different temperatures; the ion uptake anomaly may be strongly decreased by adding Ca-ion to the absorption solution; the Viets effect was observed for rice roots only above 15 °C, but for wheat already above 10 °C. Investigations with different rice root lengths (ages) show that the shorter (younger) the roots, the higher the K-ion uptake anomaly.

## Introduction

Some investigations were made earlier to study the ion uptakes of thermophilic plants (ZSOLDOS, 1968; ZSOLDOS, 1972a). It was established that for certain thermophilic plants a chilling temperature may result in an anomalous K-ion uptake and efflux (ZSOLDOS, 1974). No departure from the regular ion uptake was revealed by using $H_2PO^-_4$, $I^-$, $NO^-_3$ and $NH^+_4$ ions (ZSOLDOS, 1972b).

Our earlier data suggested that cold-treatment of cold-sensitive plants, by changing the permeability of cell membranes, stimulated passive K-ion uptake. The anomalous ion uptake could be completely prevented by adding Ca-ion to the uptake solution. At the same time, at higher temperature Ca-ion is well known to stimulate certain ion uptakes (VIETS, 1944; MARSCHNER, 1971; WALLACE, 1971; ZSOLDOS, 1974). No, or only very small changes could be observed in the anomalous K-ion uptake if uncouplers of oxidative phosphorylation (e.g. 2.4-DNP) were employed (ZSOLDOS et al., 1968).

From our earlier data it seems that the mechanism of the initial K-ion uptake anomaly, at least for thermophilic plants, is rather complicated and somewhat different from that of other plants (ZSOLDOS and KARVALY, 1975). For this reason, further studies have been carried out in connection with the low-temperature effect. In this paper attention is paid to the investigation of the effects of low and high temperatures on the K-ion uptake. In the course of these investigations special attention was given to the temperature-dependence of the Viets effect. These studies were also extended to a comparison of K-ion uptakes in rice plant at different root lengths and temperatures.

## Materials and Methods

Rice (*Oryza sativa* var. *japonica* cultivar Dunghan Shali) and spring wheat (*Triticum aestivum* cultivar Tobari 66) seedlings were grown in $5\times10^{-4}$ M $CaSo_4$ solution under well-controlled conditions as described earlier (ZSOLDOS, 1972). 6—7 cm long roots of 7-day-old plants were excised and washed in distilled water for 10 minutes at room temperature. About 3 g of the root material was placed in 500 ml aerated, isotopically-labelled uptake solutions kept at different temperatures between 0 and 30 °C, for given periods of time.

The temperature of the absorption solution was constant within 1 °C during the incubation process. The pH of the uptake solution was adjusted to 6.4—6.5. No noticeable change in pH occurred during the experiments. The uptake studies were carried out in $10^{-3}$ M K($^{86}$Rb)Cl solution. For technical reasons, $^{86}$Rb was chosen as label for potassium. Before the systematic investigations, comparative studies were performed on the differences in behaviour of K-ion and Rb-ion during uptake (ZSOLDOS and KARVALY, 1975).

The root samples were removed from the uptake solution at different intervals and rinsed three times in distilled water for one minute at room temperature. The roots were then dried as described earlier and the activites of the samples were measured by scintillation counter (ZSOLDOS, 1972). Results are given in $\mu$M/g dry weight.

In the investigations of the K-ion uptakes of rice roots with different lengths (ages), intact roots with lengths of 4, 6 and 8 cm were used. To obtain roots suitable for our experimental purposes, rice was sown every one and a half days.

All experiments were repeated at least there times. Although the absolute values were not exactly the same for individual samples, the trends were identical, showing the effects to be due to sample.treatment and not to sample differences.

## Results and Discussion

### 1. Investigation with rice roots

Fig. 1 shows the rate of K-ion uptake of excised rice roots at different temperatures and in the presence or absence of Ca-ion. It can be seen from the results that, as described in detail earlier, anomalous K-ion uptake is observable at lower temperature. This effect, however, could be strongly decreased by adding Ca-ion to the uptake solution (ZSOLDOS, 1972). At higher temperature, as can be seen from Fig. 1, the presence of Ca-ion in the absorption solution enhanced the uptake of K-ion, bringing about the well-known Viets effect.

These experimental results indicated that in the Viets effect there is a lower critical temperature, below which the Ca-ion inhibits the K-ion uptake. Our results showed that in rice seedlings the lower critical temperature of the Viets effect was ca. 14 °C. Below the critical temperature value, physiological or physical changes take place in the cell membrane, with resulting temperature injury.

In this connection we refer to the review by LYONS (1973), dealing in detail with the physiological manifestation of chilling injury. The investigations, carried out especially with the membranes of mitochondria, clearly showed that the membranes did undergo a physical-phase transition from a flexible liquid-crystalline to a solid-gel structure at 10 °C to 12 °C (LEVITT, 1972). It seems that these results are precisely correlated with the temperature below which injury occurred in the rice plant too.

### 2. Investigation with wheat roots

The K-ion uptakes at different temperatures are depicted in Fig. 2. The experimental results on wheat roots indicated primarily that no departure from the regular

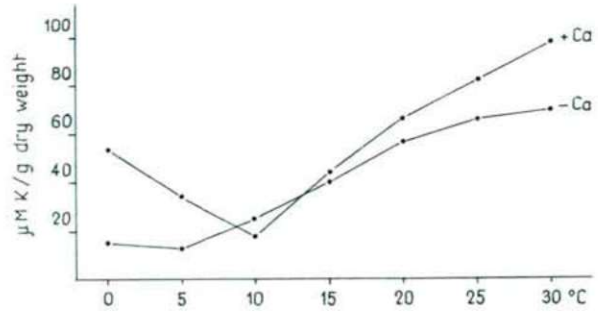



Fig. 1. K-ion uptake by excised rice from $10^{-3}$M KCl solution at different temperatures in the presence and absence of $10^{-3}$M $CaCl_2$. Uptake time: 60 minutes.

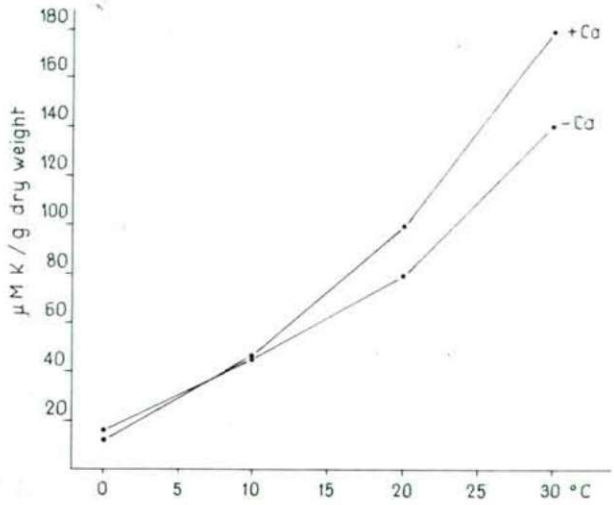



Fig. 2. K-ion uptake by excised spring wheat roots from $10^{-3}$M KCl solution at different temperatures in the presence and absence of $10^{-3}$M $CaCl_2$. Uptake time: 60 minutes.

ion uptake was revealed by using K-ion at different temperatures. It can also be stated that the lower critical temperature range of the Viets effect is considerably different from that found for rice seedlings.

The results showed that the lower critical temperature of wheat roots was ca. 10 °C. In our opinion comparison of these data with the results obtained with rice seedlings, helps to explain better the Viets effect, not satisfactorily cleared up at present (WALLACE, 1971).

### 3. Investigation with rice seedlings of different root lengths

From our earlier scattered obsservations the conclusion was drawn, that the root lengths of thermophilic plants may influence the K-ion uptake anomaly. As the problem is of great importance from a methodological point of view, we have

carried out some investigations in this direction. The results are demonstrated in Fig. 3. From the graphs it is easy to see that the K-ion uptake anomaly with the 4 cm long roots is strikingly higher than those with 6 or 8 cm long roots. At 21 °C, on the other hand, where it is well known that no uptake anomaly is experienced, there is practically no difference in the ion uptake of roots of different lengths.

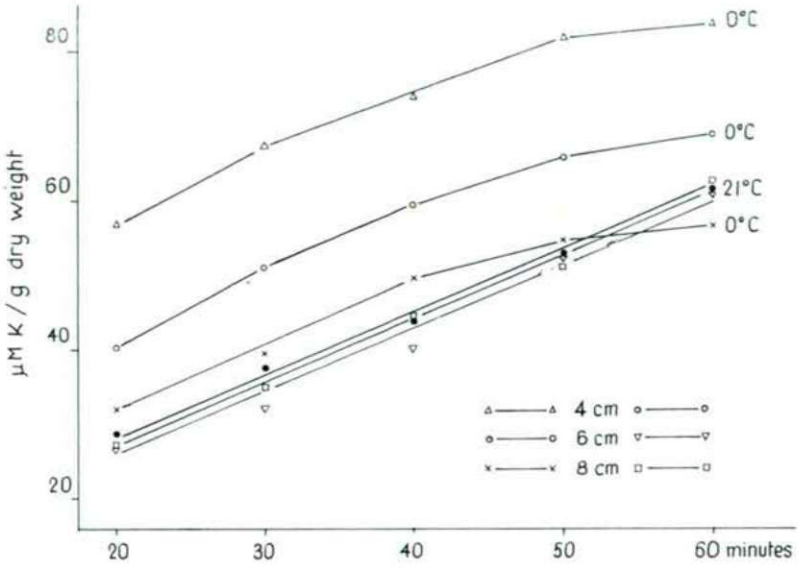



Fig. 3. K-ion uptake by excised rice roots from $10^{-3}$M KCl solution at different temperatures and root lengths.

From the above results it is obvious that in the investigation of the ion uptake of thermophilic plants one must take into consideration the fact that the extent of the uptake anomaly is strongly influenced by the length of the roots. Failing this, chiefly when we screen the cold-resistance of different varieties of thermophilic plants, we may obtain faulty results. The experimental data also indicate the need for further investigations of the K-ion uptake anomaly of root segments. These are now in progress.

## References

LEVITT, J. (1972): Responses of Plants to Environmental Stresses. New-York, Academic Press. p. 697.

LYONS, J. M. (1973): Chilling injury in plants. — Ann. Rev. Plant Physiol. *24*, 445—466.

MARSCHNER, H. (1971): Wechselwirkungen zwischen $Ca^{++}$ and Alkaliionen bei der Aufnahme durch Pflanzenzellen. In: First European Biophysics Congress, Baden near Vienna, pp. 89—95.

VIETS, F. G. (1944): Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. — Plant Physiol. *19*, 466—480.

WALLACE, A. (1971): Low root temperature, calcium and nitrate ion interactions on non-exchangeable rubidium, cesium and sodium absorption by bush beans. — Plant and Soil *34*, 121—131.

ZSOLDOS, F. (1968): Uptake of rubidium ion in different plants exposed to sudden fall in temperature. — Z. Pflanzenernähr. Düng. Bodenkunde *119*, 169—173.
ZSOLDOS, F., SIROKMÁN, F. and CSEH, E. (1968): Rubidium uptake and exchange of roots influenced by low temperature and some inhibitors. — Z. Pflanzenphysiol. *60*, 169—171.
ZSOLDOS, F. (1971): Isotope technique for investigations of cold resistance in rice and sorghum varieties. — Plant and Soil *35*, 659—663.
ZSOLDOS, F. (1972a): Ion uptake by cold-injured rice roots. — Plant and Soil *37*, 469—479.
ZSOLDOS, F. (1972b): Influence of chilling on ion uptake by roots. — Acta Biol. Szeged. *18*, 121—129.
ZSOLDOS, F. (1974): Temperature-dependence of potassium uptake in plants. — In: Potassium Research and Agriculture Production. International Potash Institute, Berne, pp. 167—174.
ZSOLDOS, F. and KARVALY, B. (1975): Effect of temperature and concentration on the anomalous potassium uptake of thermophilic plants. — Experientia 31, 75—76.

Address of the author:
Dr. F. ZSOLDOS
Department of Plant Physiology,
A. J. University,
H—6722 Szeged, Hungary

Acta Biologica Szeged. *21* (1—4), pp. 89—93 (1975)

# QUANTITATIVE BIOLOGICAL ASSAY OF MELATONIN

I. A. NAGY and B. MATKOVICS*

*Isotope Laboratory, Department of Biochemistry, Attila József University, Szeged*

(Received March 8, 1975)

**Abstract**

A quantitative calibration curve was prepared by measurement of the contraction of the melanophores on the action of commercial melatonin in two test objects, frog and newt. The upper and lower limits of the quantitative reaction were established (20 and 0.5 ng/ml).

The MT-dependent quantitative melanophore reaction was then used to determine the MT content of bovine C. P. extracts.

The data reveal that the method is very suitable for the quantitative determination of MT within the ranges indicated (the linear section of the curve). The method is simple, cheap and rapid.

Melatonin (MT)** is one of the most important hormones of the corpus pineale (C. P.) (AXELROD, 1970a; WOLSTENHOLM et al. 1971). It is known that the histological structure of the C. P. in lower-order vertebrates is similar to that of the retina (OKSCHE et al. 1969; OWMAN et al. 1970; PETIT, 1971). In this group of animals the C. P. is part of the system which (in addition to the hypophysis) controls the accommodation of the animals to light (WOLSTENHOLM et al. 1971).

In higher-oreder vertebrates the C. P. has another function, namely the inhibition and control of the sexual activity during the maturation of the animals. The observation that the skin of frogs (Amphibia) is decolorized by a bovine C. P. extract was reported by LERNER et al. (1959) and they isolated the active substance responsible for this decolorization (KERNER et al. 1960). MT induces the color changes of the skin by aggregating the melanin around the melanophore cell nuclei. The hypophysis melanin-stimulating hormone (MSH) possesses antagonist properties towards MT. MT concentrates melanin not only in Amphibia but also in Pisces (JOSS, 1973;. REED et al. 1969). MT is the most potent mediator causing the contraction of the melanophores in the skin of Amphibia. From such an aspect it is $10^5$ times more active than noradrenaline (AXELROD, 1970b). The secretion of MT follows a diurnal-nocturnal rhythm, which is of importance in the daily rhythm of many physiological functions (AXELROD, 1970b; REITER, 1973). The MT content of the C. P. is very low, and accordingly its quantitative determination demands a sensitive method.

MT can be separated from the other compounds with indole skeleton and measured spectrophotofluoromerically in a very complex method involving repeated purification. Similar limiting factors must also be reckogned with in chromatogra-

* To whom reprint requests should be sent.

** Abbreviations used: MT = melatonin (5-methoxy-N-acetyl-tryptamine); C. P. = corpus pineale; MSH = melanin-stimulating hormone; TLC = thin-layer chromatography.

phic (paper chromatography and TLC) methods, because of the preliminary purifications (LERNER et al. 1959; LERNER et al. 1960; MCISAACet al. 1964). The MT content of the C. P. can also be followed indirectly by measurement of the hydroxyindole-0-methyl-transferase activity, since this enzyme is responsible for the final step in the synthesis of MT (AXELROD et al. 1961).

## Materials and Methods

Adult animals from the following species were used experimentally to determine the MT activity: Rana ridibunda, Triturus vulgaris, Carassius auratus spec. Japonicus var. bicaudatus.

In animals from each group measurements were first made of the rest size of the melanophores, employing a CYTOPLAST stereomicroscope (PZO, Poland) a Bürker chamber and an ocular micrometer. The shape of the melanophores is extremely similar to that of the multipolar neurocytes in mammals. The areas in which the melanophores were observed were the abdominal skin in the the membrane between the toes in the newt, and the tail fin and abdominal skin in the fish. Chloral hydrate was used to immobilize the test objects.

The MT used for the preparation of the calibration curve was a Calbiochem (USA) product. Ethanolic solutions of various concentrations were prepared. These were administered to the animals by injection, diluted with known amounts of physiological salt solution if necessary. A normal scale is given in the recording of the calibration curve, where the melanophore size is indicated in mm, and the MT concentration in ng/ml (see Fig. 1).

In general 50 melanophores were observed in the visual field. Their average size is given later.

The method described here is a specific biological determination. The decrease in intensity of the colour of the skin can be observed by two methods: (a) photometrically, measuring the transparency of the skin ;(b) the shape of the melanophores and their contraction can be followed directly by microscope, and the change can be measured quantitatively. We selected the latter method for the determination of MT, since we consider it more accurate than the former (MORI et al. 1960; RALPH et al. 1970).

## Results and discussion

The size of the melanophores of the immobilized animals was measured in the rest state.

1. It was found that under normal light conditions the size of the melanophores was largest between 1 and 3 p.m.:

| | |
|---|---|
| *Carassius a.* | 0.1562—0.1704 mm, |
| *Triturus v.* | 0.0852 mm, |
| *Rana* r. | 0.0852 mm. |

2. In strong light the size of the melanophores in the fish did not change, but those in the Amphibia were more dispersed:

| | |
|---|---|
| *Triturus v.* | 0.0994—0.1136 mm |
| *Rana r.* | 0.0994—0.1136 mm |

The melanophore size was measured on maximum contraction after administration of MT. The highest MT concentration used was 30 ng/ml (see calibration curve, Fig. 1). The calibration graph reveals that even 20 ng/ml MT gives rise to a strong contraction, this not being increased further by a concentration of 30 ng/ml.

| | |
|---|---|
| *Carassius a.* | 0.0142 mm, |
| *Triturus v.* | 0.0142 mm, |
| *Rana r.* | 0.0142 mm. |

Following this, a calibration curve was prepared on new experimental objects by measuring the average size of the melanophores at different MT concentrations.

| Melatonin concentration (ng/ml) | Melanophore size (mm) |
|---|---|
| 30.0 | 0.0142 |
| 20.0 | 0.0142 |
| 15.0 | 0.0200 |
| 10.0 | 0.0282 |
| 5.0 | 0.0426 |
| 1.0 | 0.0568 |
| 0.5 | 0.0710 |
| 0.2 | 0.0750 |

The average was calculated from the frog and newt melanophore data in 10 fields of vision. (It did not prove possible to obtain a linear correlation between the MT concentration and the reaction of the pigment cells in the case of the fish species examined.)

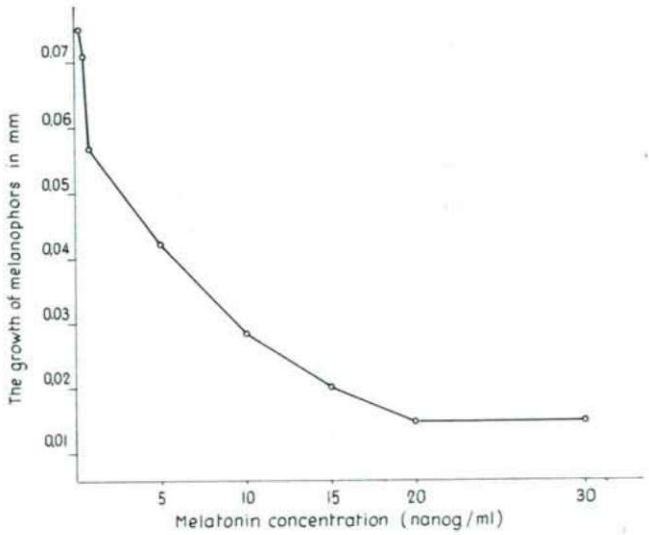



Fig 1

It can be seen from the curve that size of the melanophores does not change in the MT concentration range 20—30 ng/ml. In the range 0.5—20.0 ng/ml the curves were linear. No change in size could be demonstrated at MT concentrations lower than 0.2 ng/ml. From this the conclusion can be drawn that under normal conditions in the frog and newt 0.2—0.5 ng/ml MT maintains equilibrium with the given amount of MSH.

Subsequently, extracts were prepared from 20 bovine C. P. The extraction was performed with petroleum ether at room temperature from the homogenizate

prepared with physiological salt. The petroleum ether phase was separated and discarded (lipid extracts). The homogenizate was then extracted twice with its own volume of ethyl acetate. The ethyl acetate extracts were separated and evaporated to dryness under $N_2$ at 40 °C. The aqueous phase was reextracted in the same manner with ethyl acetate and the ethyl acetate extracts were combined and evaporated to dryness as before. The collected residue was dissolved in 1 ml dry ethanol and the quantitative determination carried out by the above detailed method.

The dry residue of the ethyl acetate extract of the lipid-free C. P. homogenizate gave rise to a melanophore response corresponding to an MT concentration of on average about 10 ng/ml per corpus pineale.

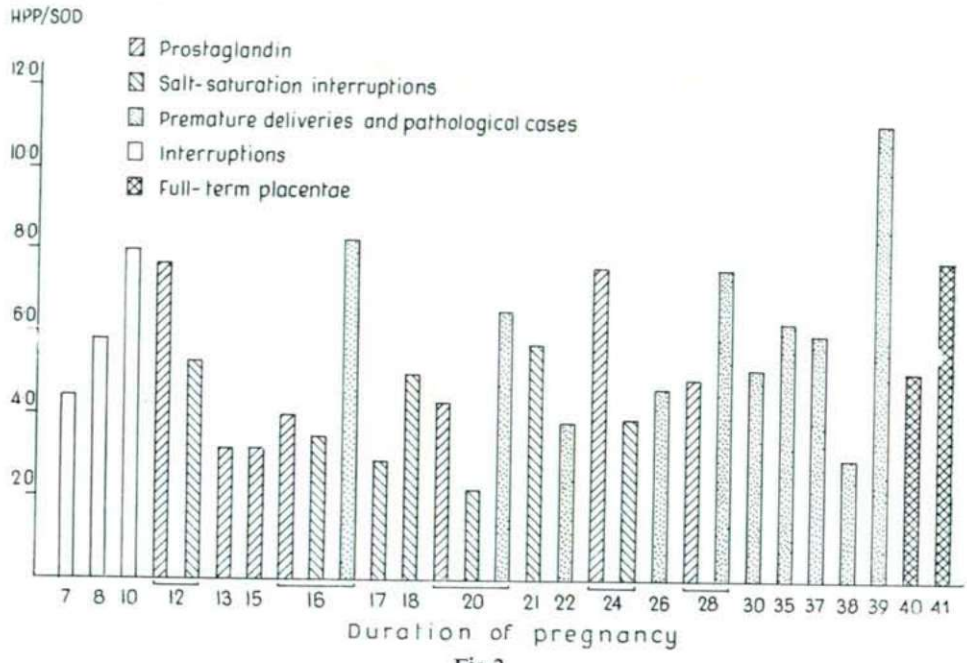



Fig 2

## References


AXELROD, I. (1970a): The pineal gland. — Endeavour *29*, 144—148.

AXELROD, I. (1970b): Comaparive biochemistry of the pineal gland. — Am. Zoologist. *10*, 259—267.

AXELROD, I., WEISSBACH, H. (1961): Purification and properties of hydroxyindole-0-methyl transferase. — J. Biol. Chem. 236 211—213.

JOSS, J. M. P. (1973): The pineal complex, melation and color change in the Lamprey Lampetra. — Gen. Comp. Endocrinol. *21*, 188—195.

LERNER, A. B., CASE, J. D., HEINZELMAN, R. V. (1959): Structure of melatonin. — J. Am. Chem. Soc. *81*, 6084—6085.

LERNER, A. B. CASE, J. D., TAKAHASHI, Y. (1960): Isolation of melatonin and 5-methoxy-indole-3--acetic acid from pineal glands. — J. Biol. Chem. *235*, 1992—1997.

MCISAAC, W. M., TABORSKY, R. G., FARNE°L, G. (1964): 5-Methoxy-tryptophanol: Effect on estrus and ovarian weight. — Science *145*, 63—64.

MORI, W., LERNER, A. B. (1960): A microscopic bioassay for melatonin. — Endocrinology *67*, 443—450.

OKSCHE, A., MORITA, Y., VAUPEL von HARNACH, M. (1969): Zur Feinstruktur und Funktion des Pinealorgans der Taube *Columba livia* . — Z. Zellforrsch. *102*, 1—30.

OWMAN, CH., RÜDEBERG, C. (1970): Light, fluorescenace and electron microscopic studies on the pineal organ of the pike. *Esox lucius* L., with special regard to 5-hydroxy-tryptamine. — Z. Zellforsch. *107*, 522—550.

PETIT, A. (1971): L'epiphyse d'un serpent: *Tropidonatus natriy* L. I.—II. — Z. Zellforsch. *120*, 94—119; 246—260.

RALPH, CH. L., LYNCH, H. I. (1970): A quantitative melatonin bioassay. — Gen. Compar. Endocrinol. *15*, 334—338.

REED, B. L., FINNIN, B. C., RUFFIN, N. E. (1969): The effects of melatonin and epinephrin on the melanophores of freshwater teleosts. Life Sci. *8*, (II) 113—120.

REITER, R. J. (1973): Comparative physiology: Pineal gland. Ann. Rev. Physiol. *35*, 305—328.

The p.neal gland. Ciba Found. Symp. Edited by G. WOLSTENHOLDME and M. O'CONNOR (1971); J. and A. CHURCHILL Ltd., London.

Address of the authors:
I. A. NAGY
Dr. B. MATKOVICS
Isotope Laboratory, Department of Biochemistry, A, J. University, H—6726 Szeged, P. O. Box 539, Hungary

Acta Biologica Szeged. *21* (1—4), pp. 95—97 (1975)

# THE LIPIDS OF THE BOVINE PINEAL GLAND
## (Short communication)

I. A. NAGY and B. MATKOVICS*

*Isotope Laboratory Department of Biochemistry, Attila József University, Szeged*

(Received March 8, 1975)

**Abstract**

The phospholipid composition and cholesterol content of 2-year-old bovine pineal gland extracts were determined. The total lipid content was 3.7—5.6% of the wet weight. In general 71% of the total lipids consists of various phospholipids, and 15% of free cholesterol.

The study of the lipids of the pineal gland was considered necessary becuase the hormone of this organ, melatonin, is a lipophilic substance (Index Merck, 1968), and because large changes take place in the mutual proportions of the lipids (mainly phospholipids) during various pathological processes (ZWEENS, 1963) and pathological changes (KAHÁN, 1971). In addition, various transmitters similarly affect the proportions of the phospholipids of the pineal gland and the uptake of $H_3{}^{32}PO_4$ ($^{32}P_i$)** (BASINSKA et al., 1973). The role of the neutral lipids and fatty acids is predominantly to act as a store of reserve nutriment, in the form of drops which can be detected histochemically in the cytoplasm of the pineal gland (QUAY, 1957; ROOZEMOND et al., 1970).

## Materials and Methods

The bovine pineal glands used in the study were obtained from the Szeged abattoir. The study material was maintained at −4 °C. It was washed in physiological salt solution and the weight of the glands measured. The weight of one gland was 0.2—0.25 g. Samples taken for examination consisted of 5—10 glands. The samples were homogenized with a cold mixture of chloroform and methanol (2:1) (FOLCH et al., 1951), and the crude extract was washed with 0.88% potassium chloride (FOLCH et al., 1957). Other samples were treated in the following way: the material was homogenized in the above-mentioned mixture, and extracted under shaking for 30 min, and the water-soluble impurities were removed by dialyzation against distilled water. The extracts were evaporated to dryness and determined gravimetrically (KREMMER et al., 1969). The evaporated lipids were dissolved in chloroform — methanol (2:1); in general a 3% solution was prepared.

For purposes of TLC, Kiesegel G nach Stahl adsorbent was applied in a thickness of 0.25 mm to 10×20 cm and 20×20 cm glass plates with the aid of a Desaga apparatus. The plates were activated at 110 °C for 20 min.

Lecithin, stearic acid, palmitic acid, cholesterol and cholesterol acetate were used as comparative standards.

The solvent used to separate the individual components of the total lipid solution was n-hexane — diethyl ether — glacial acetic acid (73:25:2). The following fractions were obtained:

* To whom reprint requests should be addressed

** Abbreviations used: $P_i$ inorganic orthophosphate; TLC thin layer-chromatography.

phospholipid, cholesterol, mono-, di- and triglycerides, fatty acids, and cholesterol esters. The plates were placed for a short time in iodine vapour, and the spots of the developed lipid fractions were marked. The lipid fractions on the chromatogram were next collected quantitatively into test-tubes (ALTHAUS et al., 1973). The individual components of the phospholipid fraction were separated further by ascending TLC on the above adsorbent.

The following solvents were employed to separate the individual members of the phospholipid fractions: chloroform — methanol — water — acetic acid (65:35:3.6:2), and chloroform — methanol — 25% ammonia (70:30:5). The separated components were collected individually and determined quantitatively, the phospholipids by the Fiske—Subbarow method (MÜHLRÁD, 1970), and cholesterol by the Lievermann—Burhard reaction (LIEBERMANN, 1885).

## Results

It was found gravimetrically that the lipid content was 3.7—5.6% of the wet weight of the bovine pineal gland. The lipid content increased with the age of the pineal gland:

| | |
|---|---|
| 1 year | 3.7%; |
| 2 years | 4.1%; |
| 3 or more years | 4.9—5.6%. |

With the above methods five phospholipids could be identified: sphingomyelin, phosphatidly inositol, phosphatidyl choline, phosphatidyl serine and phosphatidyl ethanolamine.

The cholesterol content was found to be 15% of the total lipids.

It was also established that the phospholipids make up 71% of the total lipid content. The percentage distribution of these is shown in the following Table:

*Table*

| Pineal gland phospholipid fraction | Proportion of the phospholipid component (in %) |
|---|---|
| unknown | 1.03 |
| sphingomyelin | 12.20 |
| phosphatidyl choline | 48.30 |
| phosphatidyl inositol | 5.65 |
| phosphatidyl serine | 7.91 |
| phosphatidyl ethanolamine | 19.41 |
| unknown II | 5.5 |
| total | 99.99 |
| (total phospholipid content of the extract) | 97.33 |

With the aid of the phosphate calibration curve the phosphate contents of the indiviudal phospholipid fractions were obtained in mg, and from these the amounts of the individual components were calculated in mg and in %. Phosphate determination was also carried out on the original total phospholipid solution, for the sake of comparison. The values for the individual components were added together and the result was compared with the value calculated from the phospholipid solution; it was found that the error was $\pm 1.33\%$.

## Discussion

86% of the lipids in the total-lipid extract of the pineal gland was identified. The residual 14% comprises cholesterol esters, mono- and diglycerides and waxes.

Of the components of the phospholipid fraction (which constitues 71% of the total lipids), only 6.53% could not be identified. The smaller part of this (unknown I) is situated near the TLC front, and can probably be identified as triglycerides accompanying the lipids. The larger part of it (unknown II) remains near the start point; this contains several components (cerebroside, ganglioside, etc.).

## References

ALTHAUS, H. H. and NEUHOFF, V. (1973): One-Dimensional Micro- Chromatography of Phospholipids and Neutral Lipids on Sodium Silicate-Impregnated Silica Gel Layers. — Z. Physiol. Chem. *354*, 1073—1076.

BASINSKA, J., SASTRY, P. S. and STANCER, H. C. (1973): Incorporation of P Orthophosphate into Phospholipids of Calf Pineal Slices in the Presence and Absence of Neurotransmitters. — Endocrinology *90*, 1588—1595.

FOLCH, J., ASCOLI, I., LEES, M., MEATH, J. A. and LEBARON, F. N. (1951): Preparation of lipid extracts from brain tissue. — J. Biol. Chem. *191*, 833—841.

FOLCH, J., LEES, M. and SLOANE—STANLEY, G. H. (1957): A simple method for the isolation and purification of total lipids from animal tissues. — J. Biol. Chem. *206*, 497—509.

KAHÁN, A. (1971): A vér szerepe a retina betegségeinek keletkezésében és gyógykezelésében (Doktori értekezés.)

KREMMER, T., FERENCZY, E. és PÓSCH, E. (1969): Serum lipid frakciók mennyiségi meghatározása vékonyréteg chromatographia és bikromátos oxidáció segítségével. — Kísérletes Orvostud. *21*, 538—546.

LIEBERMANN, C. (1885): Über das Oxychinoterpen. — Chem. Ber. *18*, 1803—1809.

ROOZEMOND, R. C. (1970) Phospholipid composition of some parts of rat hypothalamus, and its relation with some histochemical observations. — J. Neurochem. *17*, 179—188.

QUAY, W. B. (1957): Cytochemistry of pineal lipids in rat and man. — J. Histochem. Cytochem. *5*, 145—152.

ZWEENS, J. (1963): Influence of the Oestrus Cycle and Ovariectomy on the phospholipid Content of the Pineal Gland in the Rat. — Nature *197*, 1114.

Address of the authors:
I. A. NAGY
Dr. B. MATKOVICS
Isotope Laboratory, Department
of Biochemistry, A. J. University,
H—6726 Szeged, P. O. Box 539,
Hungary

Acta Biologica Szeged. *21* (1—4), pp. 99—111 (1975)

# PROPERTIES OF ENZYMES VII. PURIFICATION AND PROPERTIES OF HUMAN PLACENTAL SUPEROXIDE DISMUTASE, AND TOTAL PEROXIDASE ACTIVITIES IN NORMAL AND PATHOLOGICAL CASES

KATALIN KOVÁCS, D. G. SZŐNYI and B. MATKOVICS

*Isotope Laboratory Department of Biochemistry, Attila József University, Szeged*

(Received March 8, 1975)

The results achieved in connection with the purification and properties of human placental superoxide dismutase are reviewed. The rough molecular weight of the purified enzyme was determined. After the hydrolysis of enzyme protein **2** its quantitative aminoacid composition was determined and compared with data for other human superoxide dismutases. The changes in the total superoxide dismutase and total peroxidase were brought into correlation with the age of the placenta. A certain biological periodicity can be observed in the changes of activity of the enzymes in normal placentae.

As a continuation of our earlier work (PHAM VAN HIEN, 1974; MATKOVICS, 1975), the present publication deals with the determination of the molecular weight and aminoacid composition of purified human placental superoxide dismutase (SOD; EC 1.15. 1.1.) and with the changes observed in the eznyme under normal and pathological conditions, these latter being supplemented with the changes in human placental peroxidase (HPP; EC 1.11. 1.7.) under parallel conditions.

## Materials and Methods

The preparation and quantitative determination of SOD were detailed previously (PHAM VAN HIEN et al., 1974). After fractionation, precipitation, purification on a Sephadex G—75 column and concentration with Molselect G—25 (Reanal, Budapest), SOD with an activity of 2350 μmole. $min.^{-1}$ $mg^{-1}$ was obtained; this was preserved in freeze-dried form for use in further examinations.

The quantitative determination of SOD was most often performed with the adrenochrome method (MISRA et al., 1972).

The quantitative determination of SOD was most often performed with the adrenochrome

In the comparative studies the placentae were pretreated to remove haemoglobin (PHAM VAN HIEN et al., 1974), and the tissue was then homogenized in cold 155 mmole/l NaCl solution (5 ml solution per 1 g wet tissue) in a Potter glass homogenizer (PHAM VAN HIEN et al., 1974).

The supernatant obtained in the course of the preparations after ethanol-chloroform (5:1) precipitation and centrifugation was used for the determination of SOD (KEELE et al., 1971).

For the peroxidase measurements, 1 g wet placental tissue, washed free of haemoglobin, was homogenized under the above-mentioned conditions, and the enzyme was determined on various aliquots of the homogenizate after adjusment of the pH to 7.1 with phosphate buffer.

The quantitative measurement of peroxidase was carried out at 470 nm with a Spektromom 360 (MOM, Budapest) photometer, using the guaiacol method (COLOWICK et al., 1955); a total volume of 3 ml was taken and 1 cm cells were used.

(The data determined for the enzymes always refer to 1 g wet tissue.)

The normal human placenta weights reported by KLOSTERMAN et al. (1954) were used to calculate the average values from the 24th week on (see Fig. 1. and Table 1).

In the purification of the SOD the protein content of the fractions was determined spectrophotometrically at 280 nm or by the LOWRY method (LOWRY et al., 1951), using bovine albumin to prepare the standard curve.

A Spektronom 301 (MOM, Budapest) spectrophotometer was employed for measurements in the UV region.

The absorption spectrum of the pure placental SOD was recorded in a 50 mmole/l potassium phosphate buffer (pH 7.8) at a protein concentration of 0.12 g/ml ,with a Unicam SP—800 spectrophotometer (Fig. 3).

Molecular weight determinations were performed on 20×20 cm plates coated with 0.6 mm layers of Sephadex G—100 superfine gel. 155 mmole-l NaCl solution was used for running in an ascending system, in a specially-designed apparatus (Pharmacia AG, Uppsala, Sweden) (Pharmacia booklet, 1971). The enzymes and comparison materials were dissolved in distilled water, dropped onto the plates, and subjected to running for about 4 h. The anzyme spots were transferred from the layer onto Whatman MN 214 paper with the replication technique (RADOLA ,1968); on the paper they were stained with 2 g/l (2.86 mmole/l) bromocresul green solution in alcohol-acetic acid (955:5), and fixed in ammonia vapour (Fig. 4).

The freeze-dried enzyme was used for the quantitative determination of the SOD protein aminoacids. Hydrolysis was achieved with 6 mole-l HCl for 22 h at 120 °C. The hydrolyzate was evaporated to dryness, and an amount equivalent to 0,1 mg protein was transferred to the column.

A Bio-Cal BC—200 aminoacid analyzer (Bio-Cal, Richmond, California, USA) was used, with a 52.0×0.9 cm column packed with Aminex A—6 (12—15 m) spherical resin (Bio-Rad Labs., Richmond California, USA). (The one-column technique was used.) The resin column was maintained at 52—53 °C throughout the analysis (Fig. 5, Tabl. 2). (The data in the Table give the number of aminoacids per protein molecule.)

The human placentae were obtained from the Department of Obstetrics and Gynaecology, University Medical School, Szeged. They originated in part from normal births, premature births and artificial abortions, or from interruptions brought about by intraamnial hypertonic sodium chloride solution and by administration of 25 mg prostaglandin $F_{2alfa}$ (Upjohn, USA). (The reasons for the interruptions were at times other pathologic processes, which are mentined separately.)

## Results and Discussion

Figure 1 and Table 1 contain values and average values for which primarily the data of KLOSTERMAN et al. (1954) were used.

*Table 1*

| Duration of pregnancy (in weeks) | Weight of child (in g) (Only the minimum and maximum values reported in the literature are given) | Weight of placenta (in g) | Average weight of placenta (in g)* | Blood-free weight of placenta (in g) |
|---|---|---|---|---|
| 8 | 4 | 8 | 8 | |
| 12 | 14—35 | 33 | 33 | |
| 16 | 55—150 | 70 | 70 | |
| 20 | 250—316 | 120 | 120 | |
| 24 | 600—672 | 195—258 | 226 | |
| 28 | 1000—1200 | 275—309 | 292 | |
| 32 | 1500—2115 | 365—483 | 424 | |
| 36 | 2200—2783 | 470—536 | 503 | 306 |
| 40 | 3000—3405 | 429—600 | 414 | 347 |

* For calculations the average values of placentae were always used.

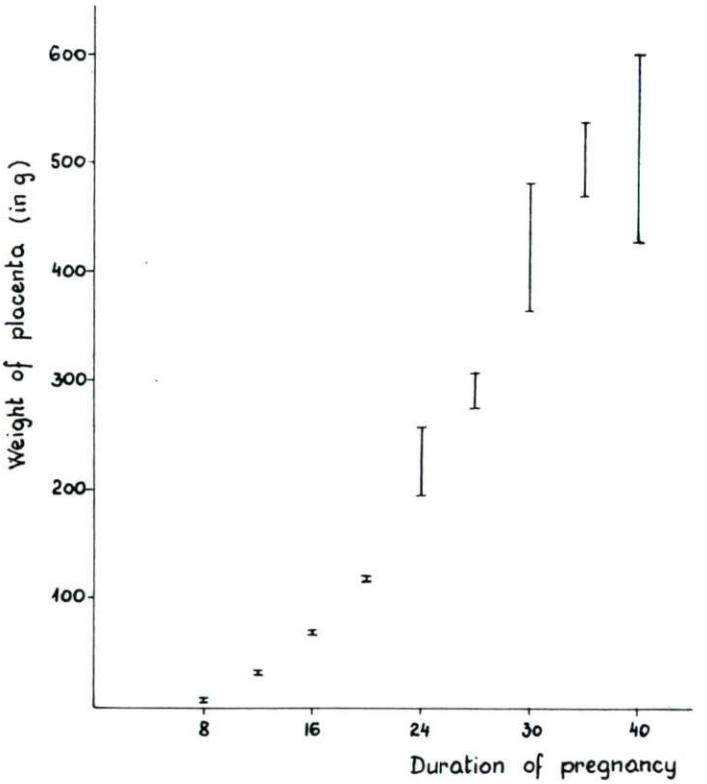



Fig. 1. Literature values illustrating average weight of placenta as a function of duration of pregnancy (KLOOSTERMAN, 1954).

Figure 2, similarly taken from the literature, is designed to show components of the full-term placenta (GARROW, 1970). Naturally, other references too could be mentioned in this respect (GELLÉN, 1969; HAMILTON, 1970).

The other Figures originate from our own experimental work. Figure 3 presents the absorption spectrum of purified human placental SOD (the details of the spectrum-recording are given in the "Materials and Methods" section).

Figure 4 illustrates the comparative SOD molecular weight values obtained with the replica technique. The comparison was performed with human and bovine erythrocyte SOD of known molecular weight. In addition, the SOD of unknown weight prepared from the full-term placenta was also depicted graphically. (Our molecular weight determinations can be said to be approximate only.)

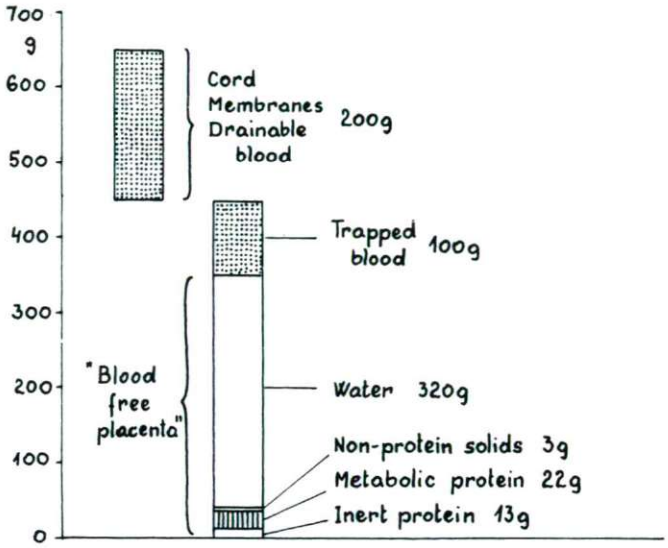



Fig. 2. Literature column-graph illustrating the main components of a full-term placenta (GARROW, 1970).

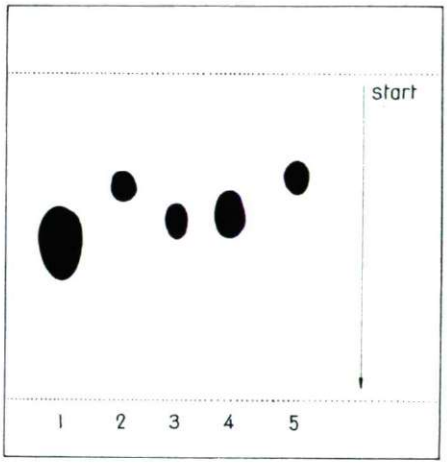



Fig. 3. UV absorption spectrum of human placental superoxide dismutase.

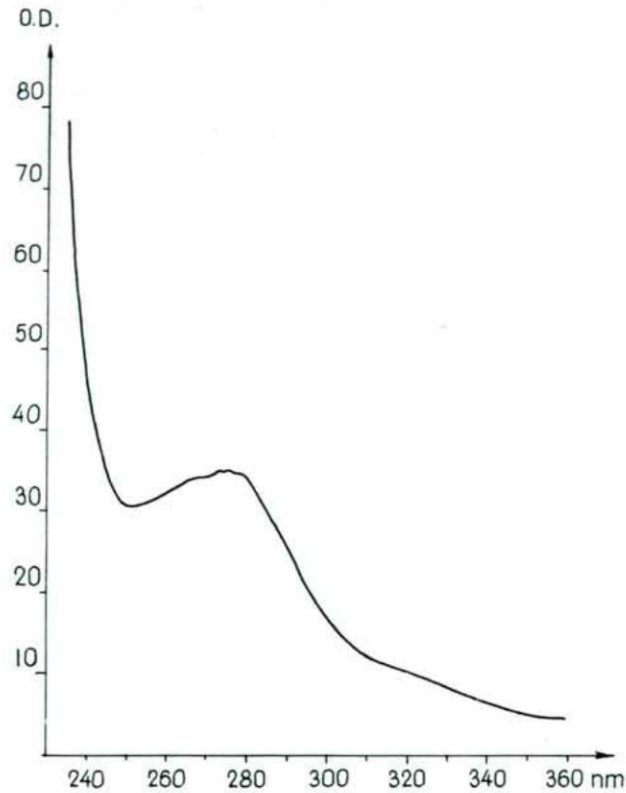



Fig. 4. Determination of molecular weight of human placental superoxide dismutase by replica techniquw

1. Pea SOD (M.W. 31,500)
2. Bovine erythrocyte SOD (M.W. 32,600)
3. Human placental SOD
4. Human placental SOD
5. Human erythrocyte SOD (M.W. 33,600)

Figure 5. presents the recorded aminoacid qualitative and quantitative values. These correspond to the data in Table 2, where, however, the quantitative data for the aminoacids were calculated via the molecular weights determined by ourselves. These permit a good comparison as regards the quantitative values for the similar human and animal (vertebrate) SOD-s

In the following three Figures (Fig. 6—8) a comparison is made of the SOD values of placentae of different ages, calculated on the total weight. It can be seen that the relative and absolute amounts of SOD increase during the development of the placenta. The Figures clearly reveal the repid rise in the SOD values of placentae developing under normal conditions. (This may be related with the protective

*Table 2*

| Aminoacidis | Bovine erythrocytes (Kovács, 1974) | Human erythrocytes (Mccord, 1971) | Normal human placentae |
|---|---|---|---|
| | M. W. 32,600 | 33,600 | 32,000 |
| Lys | 22 | 22 | 22 |
| His | 16 | 16 | 16 |
| Arg | 7 | 10 | 5 |
| Asp | 36 | 35 | 35 |
| Thr | 16 | 26 | 19 |
| Ser | 20 | 20 | 21 |
| Glu | 26 | 24 | 50 |
| Pro | 10 | 14 | 29 |
| Gly | 50 | 50 | 23 |
| Ala | 20 | 21 | 21 |
| Val | 28 | 28 | 26 |
| Met | 0 | 0 | 3 |
| Ile | 16 | 17 | 8 |
| Leu | 17 | 20 | 25 |
| Tyr | 0 | 2 | 5 |
| Phe | 8 | 10 | 12 |
| Trp | 2 | 0 | 0 |
| Cys/2 | 7 | 6* | 2 |

* from Hartz, 1972.

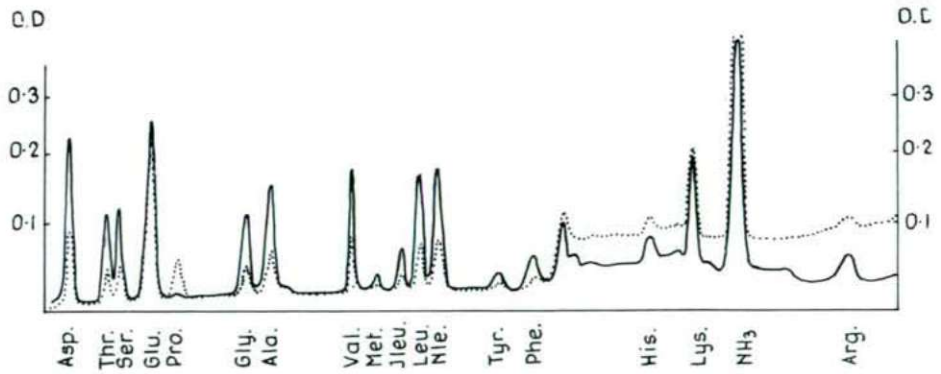



Fig. 5. Quantitative aminoacid chromatogram for human placental SOD hydrolyzate

effect of SOD, much mentioned in the literature, which tends towards the elimination of the $O^{-}_{2}$ anion (Fridovich, 1972). (See mainly Fig. 6)

If the normal development is disturbed by anything at all, or if abortion is initiated with intraamnial hypertonic sodium chloride, or prostaglandin, for instance, the measured SOD value falls considerably. Our findings refer particularly to those cases when foetal necrosis and other pathological processes (mola hydatidosa, etc). necessitate induced abortion. (Mainly sthe data of Figure 7. are of such origin.)

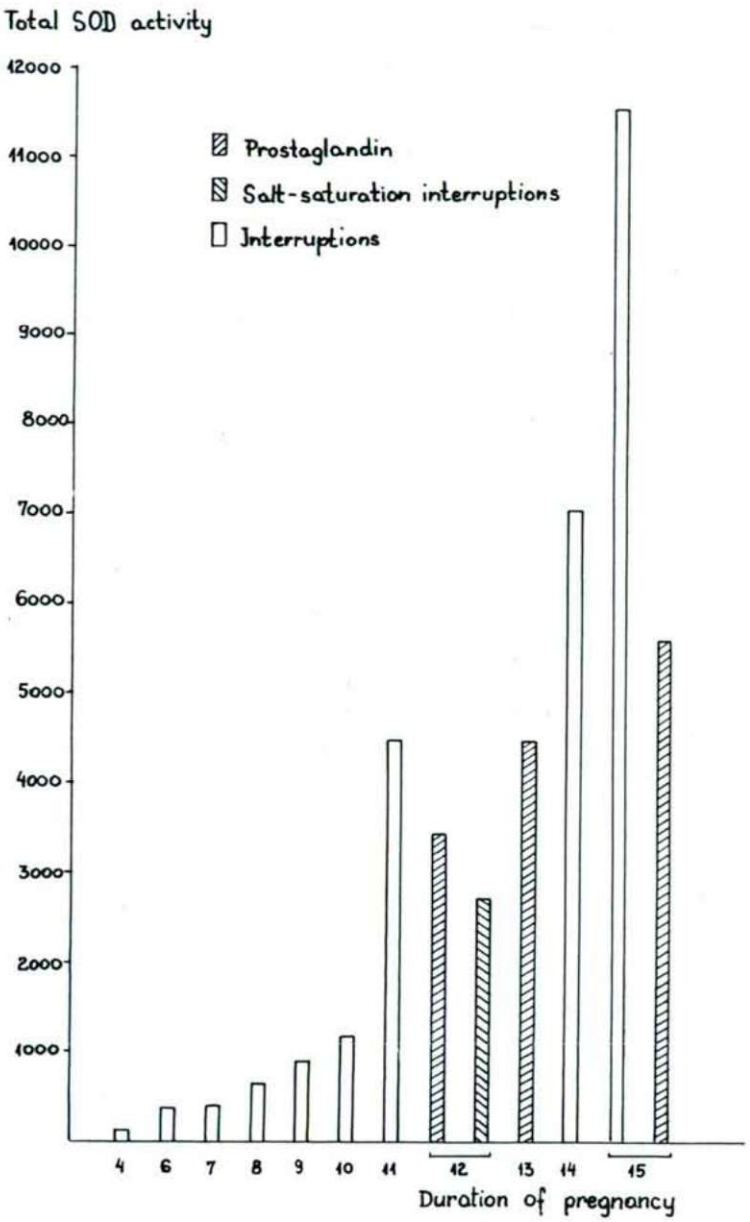



Fig. 6. Variation of total SOD activity of 4—15-week placentae. (Material originating from artificial, prostaglandin and salt-saturation abortions.)

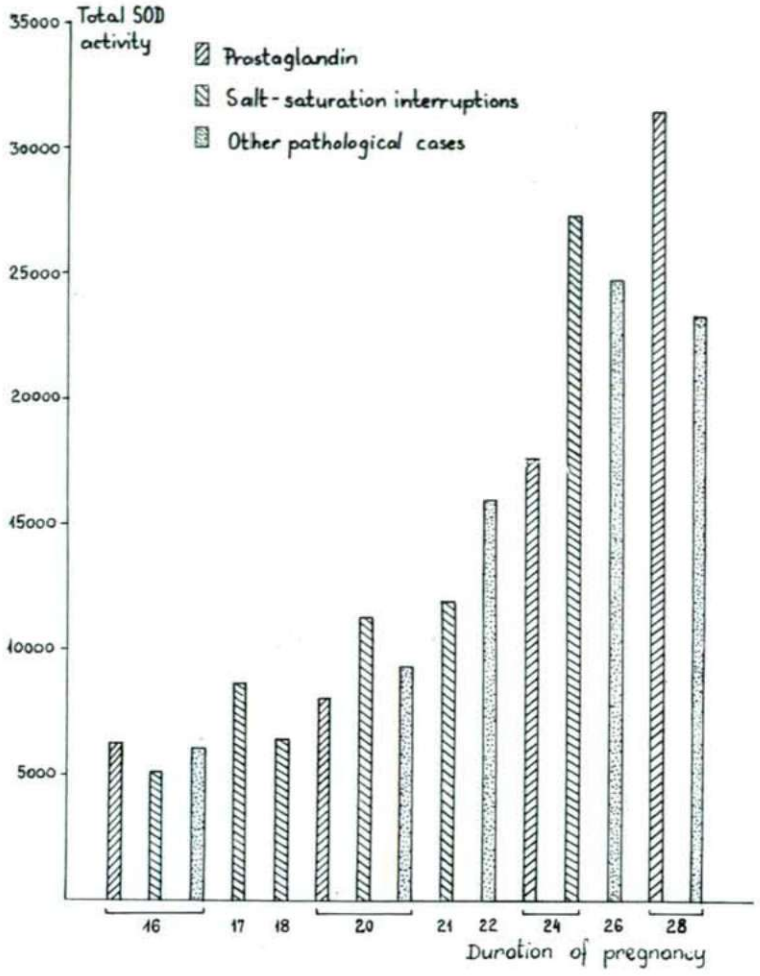



Fig. 7. Variation of total SOD activity of 16—28-week placentae. (Material originating from prostaglandin, salt-saturation and other pathological interruptions.)

(The SOD activity values in the Figures were obtained by taking the SOD values for 1 g wet placenta after the preparation detailed in the "Materials and Methods" section and multiplying by the placental weight data in Table 1).

In Figure 8 the SOD values of the full-term placentae are compared with the SOD values of placentae originating from premature deliveries.

Figure 9. compares the total SOD and total peroxidase values for placentae from artifical interruptions (4th—15th week) and full-term placentae (40th—41st week).

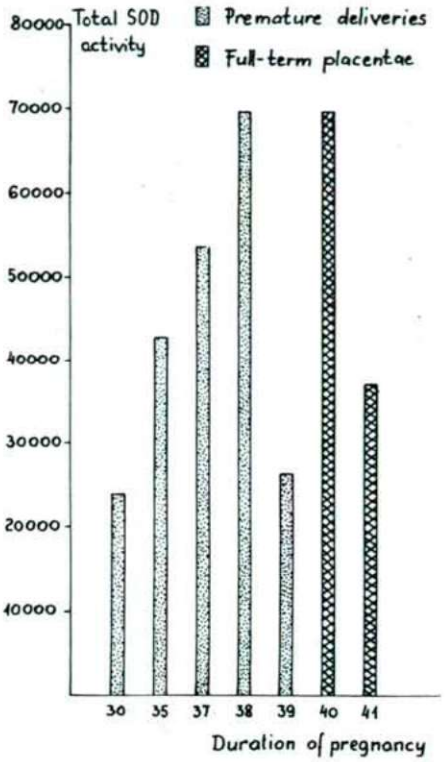



Fig. 8. Variation of total SOD activity of 30—41-week full-term placentae and placentae from premature deliveries.

Figures 10. and 11 present similar enzyme data for 12—28-week placentae. Figure 10. shows separately the total enzyme values for the prostaglandin-initiated interruptions, and Figure 11 those for the interruptions induced with hypertonic solution.

Figure 12. was obtained by dividing the total SOD value by the peroxidase value; thus, the ratio of the peroxide producing and decomposing enzymes could be characterized by values in the range 0.1—0.5 SOD produces $H_2O_2$ by the reaction:

$$2\,O^-_2 + 2\,H^+ \rightarrow H_2O_2 + O_2$$

while HPP decomposes the $H_2O_2$:

$$XH_2 + H_2O_2 \rightarrow 2\;H_2O + X_{oxide}$$

The radicals produced in the reactions can participate in many processes, of course, and may result in the formation of useful or harmful metabolites.

Figure 13. shows the reciprocal peroxide metabolism index. In this case the total HPP values were divided by the total SOD values. The values in Figure 13.

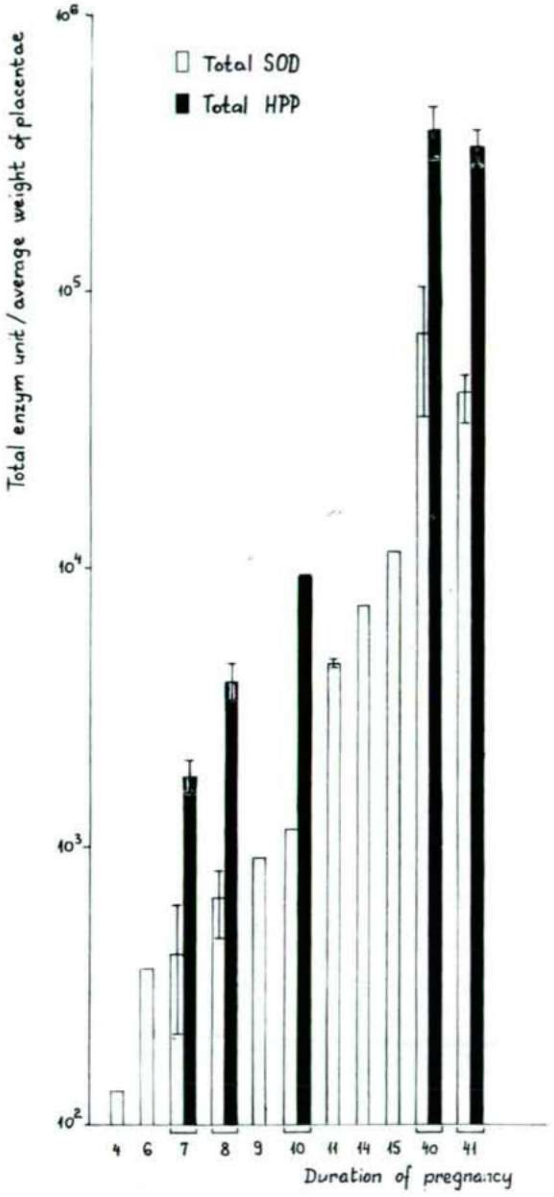
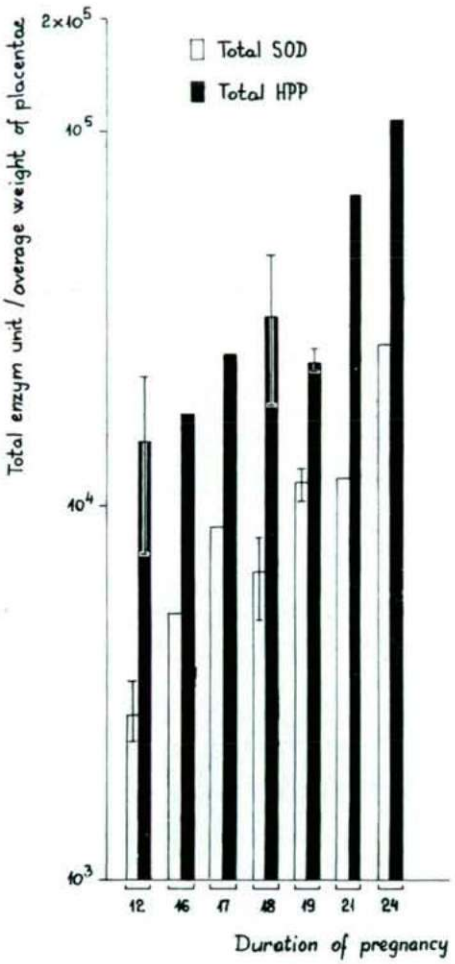



Fig. 9. Comparison of total peroxidase and total SOD activities in artificial interruption and full-term placentae.




F.g. 10. Comparison of total peroxidase and total SOD activities in material from prostaglandin interruptions.

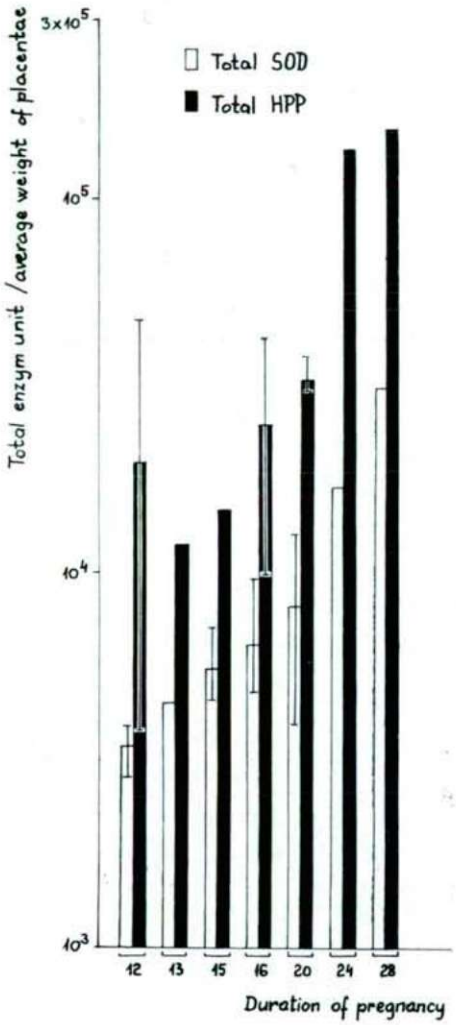



Fig. 11. Comparison of total peroxidase and total SOD activities in material from saturated-salt solution iniciated interruption cases.

lie between roughly 2 and 12; this is important perhaps from the aspect that the values are spread out more.

Both column-graphs display a certain harmonic fluctuation with regard to the two enzymes of the peroxide metabolism, reflecting a characteristic biological rhythm.

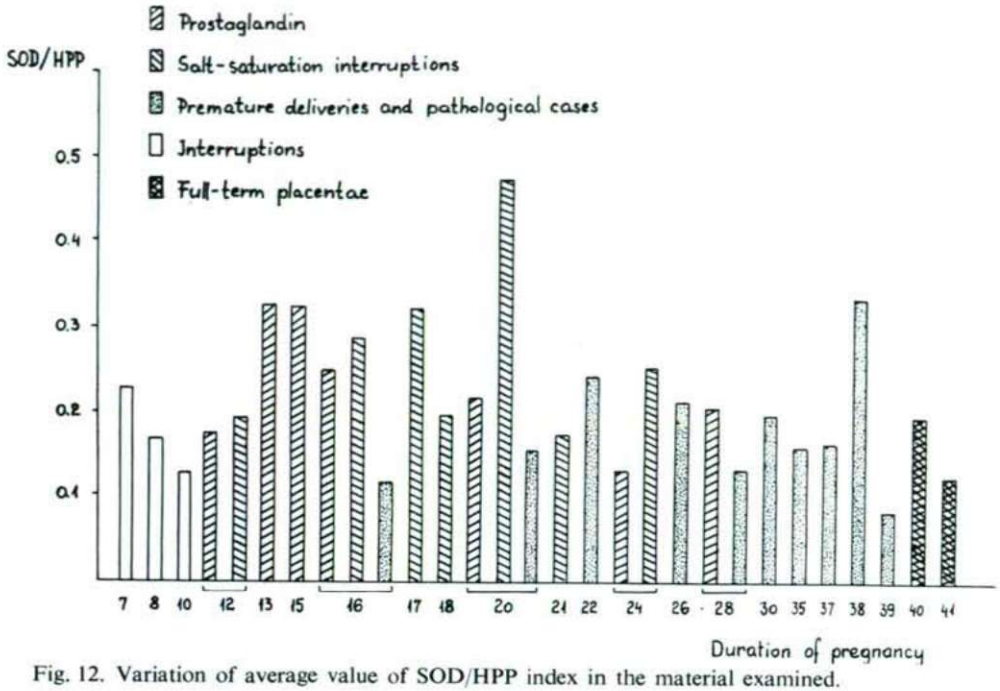



Fig. 12. Variation of average value of SOD/HPP index in the material examined.

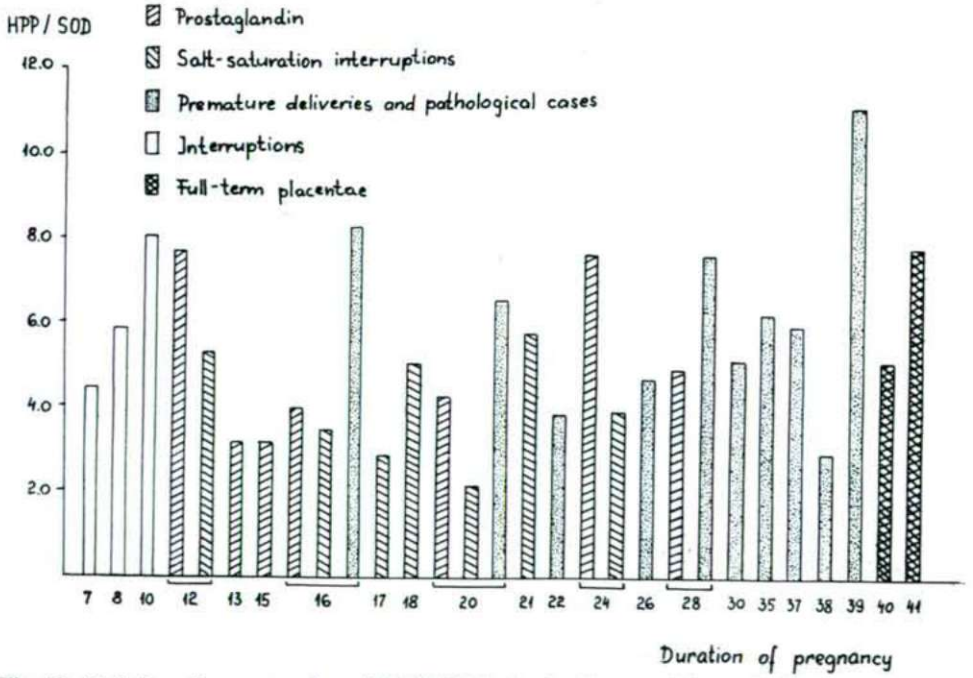



Fig. 13. Variation of average value of HPP/SOD index in the material examined.

## References

COLOWICK, S. P. and KAPLAN, N. O. (1955): Methods in enzymology, *2*, 764—775 (Academic Press, New York).

FRIDOVICH, I. (1972): Superoxide radical and superoxide dismutase. — Accounts Chem. Res. *5*, 321—326.

GARROW, J. S. (1970): The relationship of foetal growth size and composition of the placenta. — Proc. Roy. Soc. Med. *6D*, 498—500.

GELLÉN, J. and GYŐRI, I. (1969): The weight increase of the placenta and ambryo in early human pregnancy. — J. Obstet. Gynaec. Brit. Cwlth. *76*, 990—992.

HAMILTON, W. J. and GIRMES, D. H. (1970): Growth relationship between the foetus and the placenta. — Proc. Roy. Soc. Med. *63*, 496—498.

HARTZ, J. W. and DEUTSCH, H. F. (1972): Subunit structure of human superoxide dismutase. — J. Biol. Chem. *247*, 7043—7050.

KATALIN KOVÁCS (1974): Ph.D. Thesis Szeged

KEELE, B. B., jr.; MCCORD, J. M. and FRIDOVICH, I. (1971): Further characterization of bovine superoxide dimutase and its isolation from bovine heart. — J. Biol. Chem. $264_1$ 2075—2080.

KLOOSTERMAN, C. J. and HUIDE KOPER, B. I. (1954): The significance of the placenta in "obstetrical mortality". — Gynaecologia *138*, 529—550.

LOWRY, O. H.; ROSEBROUGH, N. J.; LEWIS FARR, A. and RANDALL, R. J. (1951): Protein measurement with the Folin phenol reagent. — J. Biol. Chem. *193*, 265—275.

MATKOVICS, B.; FODOR, I. and KOVÁCS, K. (1975): Properties of enzymes III. A comparative study of peroxide-decomposing enzymes and superoxide dismutase of human placenta at different ages. — Enzyme *19*, 285—293.

MISRA, H. P. and FRIDOVICH, I. (1972): The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. — J. Biol. Chem. *247*, 3170—3175. USA *68*, 1024—1027.

PHAM VAN HIEN, KOVÁCS, K. and MATKOVICS, B. (1974): Properties of enzymes I. Study of the superoxide dismutase activity change in human placenta of different ages. — Enzyme *18*, 341—347.

PHAM VAN HIEN, KOVÁCS, K. and MATKOVICS, B. (1974): Properties of enzymes II. Comparative study of superoxide dismutase activity in rat tissues. — Enzyme *19*, 1—4.

Thin-layer gel filtration with the Pharmica TLG-apparatus (Pharmacia Fine Chemicals AB, Uppsala, Sweden)

RADOLA, B. J. (1968): Thin-layer gel filtration of proteins. — J. Chromatogr. 38. 61—67.

Address of the authors:
KATALIN KOVÁCS
D. G. SZŐNYI
Dr. B. MATKOVICS
Isotope Laboratory, Department
of Biochemistry, A. J. University,
H—6726 Szeged, P. O. Box 539,
Hungary

Acta Biologica Szeged. *21* (1—4), pp. 113—125 (1975)

# THE EFFECT OF TERTIARY TROPINE DERIVATIVES ON CEREBRAL CONVULSIONS

O. FEHÉR, G. A. BÁLINT and Á. BALOGH

*Department of Animal Physiology, Attila József University, Szeged*

(Received March 25, 1975)

**Abstract**

The anticonvulsive effects of tertiary tropine derivatives were studied on pharmacological and electrical seizures of anaesthetized cats and on electroshock of waking rats. Six out of twenty compounds examined, proved to be effective on these tests. The significant anticonvulsive potency of diphenyl-acetic and Xanthen-9-carbonic derivatives of tropine deserve special attention.

During our previous experiments (FEHÉR, HALÁSZ, MECHLER, 1965) it was shown that the atropine applied to the cortex abolished the rhythmic after-discharges produced by acetylcholine and eserine, but had no effect on the strychnine potentials. From this fact the conclusion was drawn that in the mechanism of the rhythmic after-discharge cholinergic synaphes may play a role; the acetylcholeni may have an excitatory effect, or perhaps due to an inhibitory action it may enhance the activity of excitatory circuits.

Starting from these results it was examined whether the teriary derivaties of tropine which did not contain tropic acid have any inhibitory effect on the rhythmic after-discharge produced by acetylcholine and eserine, or not?

Our experiments had two main purposes. First, the anticonvulsive effect of atropine had to be controlled by the examination of some of its derivatives and on the other hand, we speculated, that these drugs having proved to be effective on the model convulsive phenomenon, may serve perhaps as new tools in the therapy.

For the investigations 20 tropine derivatives were collected, which had a minimal peripheral ganglionic inhibitory, analgesic and antitremorin effect. Thus the unwated sideeffects could be avoided, and the problems in the interference among different drugs can be reduced to a minimum. For many purposes the tertiary derivatives seem to be most suitable beacuse in case of intravenous application those derivatives have a chance to pass through the blood-brain barriere.

In Hungary NÁDOR and his coworkers (1958) produced tropine derivatives in large series, — and among their materials were found compounds which meet the requirements mentioned above.

A common feature of these derivatives was that the tropine remained either unchanged in them, or its nitrogen-atom was substituted by ethyl- or propyl-groups instead of methyl moiety. Thus our compounds to be examined differed among each other mainly in their acid constituents.

The chemical structures are listed in Table 1.

*TABLE 1*

| | | | | |
|---|---|---|---|---|
| *NA 181*<br>3-α-phenylacetoxi tropine | 0,003 | 0,05 | 0,001 | ∅ |
| *NA 184* | | | | |
| *NA 184*<br>3-α-diphenyl-acetoxi-tropine | 0,002 | 0,045 | 0,01 | ∅ |
| *NA 668*<br>3-α-(4 clorid)-bensoiloxi-tropine | 0,006 | 0,006 | 0,15 | ∅ |
| *NA 676*<br>3-α-diphenly-acetoxi-N-etyl-nortropine | 0,006 | 0,10 | 0,18 | 0,075 |
| *N 919*<br>3-α-cyclopropylcarbonyloxi-tropine | 0,001 | 0,013 | 0,06 | 0,057 |
| *N 947*<br>3-α-(9 xanthen)-carbonyloxi-N-ethyl-nortropine | 0,01 | 0,021 | 0,173 | ∅ |

*$LD_{50}$ values*
(acute mouse-toxicity)

| | | | |
|---|---|---|---|
| NA 676 | 71 mg/kg | NA 184 | 108 mg/kg |
| NA 668 | 80 mg/kg | N 919 | 112 mg/kg |
| NA 181 | 105 mg/kg | N 947 | 113 mg/kg |

Of the 20 derivatives subjected to experimental proof only those are reported in this paper which exhibited anticonvulsive action of measurable size.

The effect of the tertiary tropine derivatives was investigated from three points of view:

1. The effect on the rhythmic after-discharge produced by strychnin-neacetylcholine-eserine in the cat's cerebral cortex.
2. The effect on electric convulsions produced by direct epicortical stimulation.
3. The effect on the electroshock of waking rats.

The experiments mentioned under paragraphs 1 and 2 were carried out on cats, superficially anaesthetized by sodium-pentobarbital (40 mg/kg).

The acute toxicity of all derivatives was tested on mice. The toxicity data are given as an addendum to Table 1.

The pharmacological data of six derivatives found to be effective were taken from DECSI's candidate-thesis (1966).

Considering the fact that in some cases also cardiac-effects were seen in the first and second series of the experiments the ECG was registered, too.

The action of the experimental compounds were compared on superficially narcotized cats with a few other drugs, already being used in the therapy to detect any difference in the effect on the chemical and electric conculsions.

The solutions of the therapeutics were made from pure substances obtained directly from the factories, without any substance added.

## Methods

### Electrocorticography on cats

The animals of both sexes, weighing 1,5—3,00 kg were anaesthetized with 40 mg-kg sodium-pentobarbital given intraperitoneally. One of the femoral veins and the trachea were cannulated. The head of the animal was fixed in a stereotaxic apparatus (Type KOVÁCH) and the brain was

widely exposed on both sides; 2 or 3 ball tipped silver electrodes were placed on each hemisphere and connected to the input of a Galileo Polyphysiograph. Later series of experiments were carried out by use of an eight channel EMG Electroencephalograph. The time constant of the EEG amplifiers was 0,3 sec with an upper limiting frequency of 150 Hz.

One channel of the EEG apparatus served to record the EEG of the animal in the second lead of Einthoven.

Electroshock. Measurement of anticonvulsive activity.

For provoking electroshock on waking rats we used the method of TOMAN, SWINYARD and GOODMAN (1946). The current intensities necessary for provoking maximal seizures were determined on 45 rats. Testing electroshocks were applied in every 48 hours with gradually elevated current intensities. After a 2 week period of testing each animal showed a stable, well reproducible seizure threshold. The scattering of threshold values did not exceed 3 percent. At current strengths of 16—26 m the latency of seizures equalled 3 seconds.

Electric shocks were applied by means of bitemporal electrodes fed from a shocking-device.

After having determined seizure thresholds, the animals were divided into groups; each of them consisted of 5—7 animals. Before administration of the substances to be tested we controlled the seizure threshold by electroshocks given with 48 hours intervals. The dose of the substances was in each case 10 mg/kg body weight given intraperitoneally. 1, 4 and 24 hours after the injection we gave test shocks and the seizure intensities exhibited at these times were compared with the control ones. In cases of substances having long lasting action we made shock tests also after 4—7 days. The complete abolition of seizure we took as an effect of 100 percent, partial seizures were judged to be of 50 or 25 percent according to their intensity. If test shocks produced maximal seizure, the drug action was taken zero. From the values of a group we calculated the mean and this characterized the effectiveness of a substance at a given time after the injection.

Direct stimutation of the brain hemispheres of anaesthetized cats was made as follows. On the exposed medial ectosylvian gyri we placed silver wire stimulating electrodes bilaterally. Square wave pulses used for stimulation had the following parameters: 100 volts, 100 Hz, frequency, 0,7 msec duration. A stimulation period with the above parameters lasted for 5 seconds. At the end of stimulation EEG recording was stharted. The electroshocks provoked in this way had a duration of 15—40 seconds. If the stimulating electrodes were placed on the same side or if we p erformed high decerebration previously no seizure could be obtained.

Provocation of rhythmic after-discharge by pharmacological agents. We placed on the anterior suprasylvian gyrus a piece of filter paper (2×2 mm) soaked in 1% strychnine solution. After the appearance of the strychnine potentials another piece of filter paper was applied to the cortex, soaked in a solution containing 1 percent acetylcholine chloride and 0,1 percent physostigmine sulphate. Under such conditions a rhythmic after—discharge developed in 3—10 minutes, except the anaesthesia was too deep. The drugs tested were given intravenously in this case, too.

## The action of tertiary tropine derivatives on the after-discharge elicited pharmacologically

Fig. 1. shows the normal electrocorticogram of a cat in light anaesthesia. The respective locations of the four monopolar leads are indicated in the Figure. The common indifferent electrode was on a frontal skin flap. In Fig. 2. a typical after-discharge is visible provoked pharmacologically in the suprasylvian gyrus. A weak transcallosal projection of seizure is to be seen.

*NA 181.* The effect of 1,5 mg/kg NA 181 can be seen in Fig. 3. on a rhythmic after-discharge. This dose was capable to abolish an after-discharge of middle intensity in 9 minutes.

The fast rhythmic components disappear completely, only strychnine potentials remain. No substantial change in the electrographic picture of other areas is present.

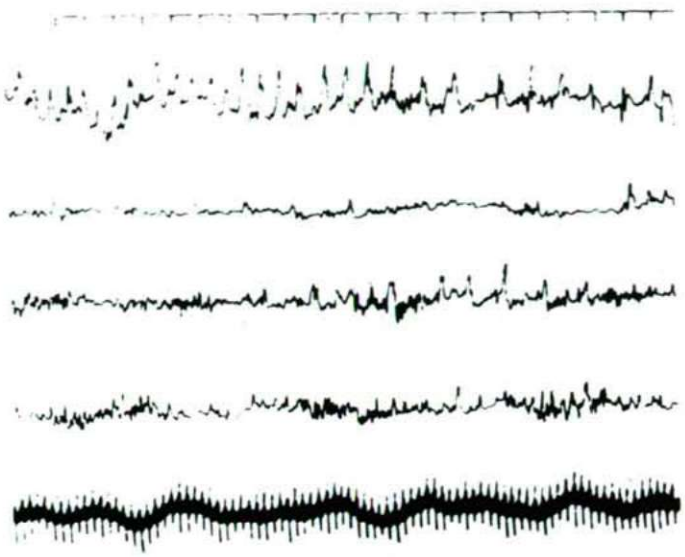


Fig. 1. Electrocorticogram of a cat anaesthetized by 40 mg-kg sodium-pentobarbital. Leads: I.: g. suprasylvius anterior left side, I.: g. ectosylvius medius, left side III.: g. suprasylvius ant. right side, IV.: g. ectosylvius medius right side, V.: Electrocardiogram in II. Einthoven lead. Time marking: 1 s.

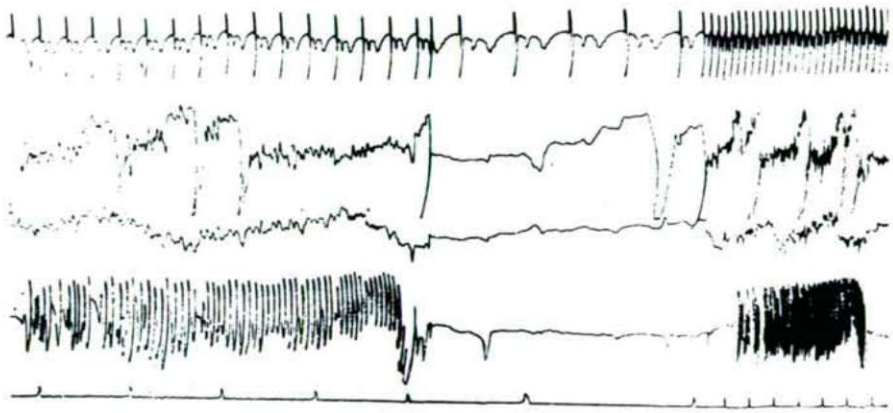


Fig. 2. Rhythmic after-discharge elicited by local application of 1 p.c. Strychnine, 1 p.c. Acetylcholine, and 0,1 p.c. Eserine solutions, on the g. suprasylvius ant, Paper speed at right is higher.

*NA 184*. The nearest relative of the former compound suppressed rhythmic after-discharges in somewhat higher doses (not illustrated).

*NA 668*. In Fig. 4. the action of a 2,5 mg/kg dose of NA 668 is demonstrated on a rhythmic after-discharge involving the anterior suprasylvian gyrus. Under drug action the after-discharge was curtailed remarkably, its frequency decreased

Fig. 3. The effect of 1.5 mg-kg NA 181 on rhythmic after-discharge provoked pharmacologically. At left control, at right 9 minutes after the injection.

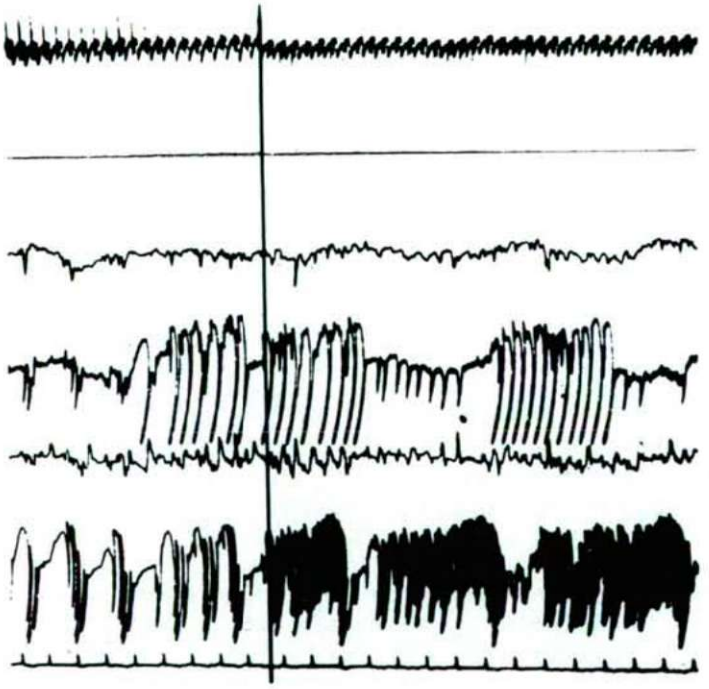


Fig. 4. Rhythmic after-discharge; 2.5 mg-kg NA 668. 5 minutes after injection.

and as only remnants, facilitated strychnine potentials could be seen. The projected strychnine spikes on the opposite side remained intact, too. In the electrocardiogram, there appeared an accentuation of the S-wave.

*NA 676.* Its effectiveness and type of action was very like to that of NA 668. A dose of 3 mg/kg reduced the after-discharge vigorously in 8 minutes (Fig. 5).

*NA 919.* Its anticonvulsive activity was weakest of all and 10 mg/kg doses were needed for suppression of after-discharges. The action consisted mainly in the curtailment of the seizure with preservation of isolated strychnine spikes.

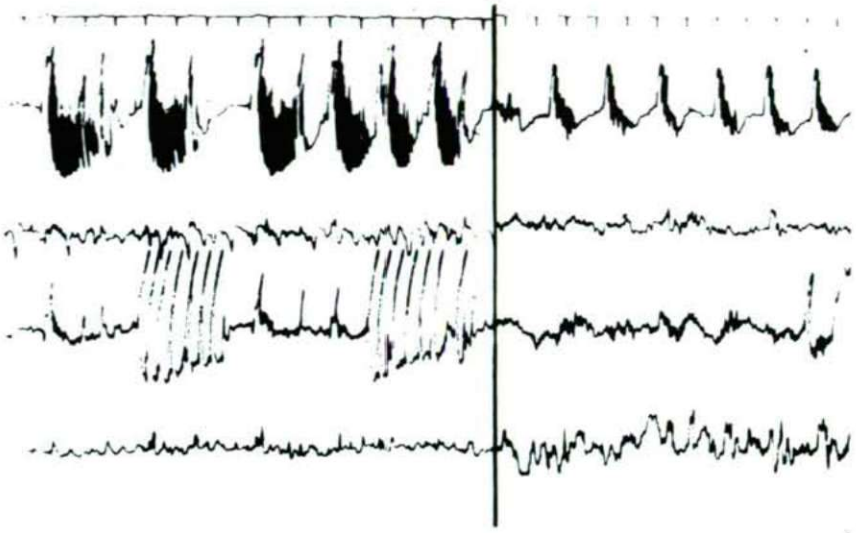


Fig. 5. Rhythmic after-discharge; 3.0 mg/kg NA 676 8 minutes after injection.

*N 947.* was one of the most potent compounds. The intravenous injection of 1 mg/kg abolished a rhythmic discharge having a projection to the opposite side, too. No remarkable cardiac effects were seen of it.

After this brief demonstration of the actions exerted on rhythmic after-discharges an important comment must be added. Our test-method used in these experiments raised rather rigorous criteria against the drugs tested. One has to consider, that substances provoking seizure discharges were present on the cortex in concentrations ranging from 0,1 to 1,0 percent. (1—10 mg/ml). The compounds tested, given intravenously in 1,5—10,0 mg/kg doses could attain a local concentration in the cerebral tissue not exceeding 1/100—1/1000th of that of the convulsive drugs. Thus they were in a 100—1000-fold drawback as related to the convulsive agents.

**Tha action of tropine derivatives on the cortical seizure phenomena elicited by direct stimulation**

In the next series of experiments electric convulsions were provoked on the cerebral cortex by means of stimulating electrodes placed on the medial ectosylvian gyri of both sides. The parameters of the stimuli were listed in the Methods. During stimulation the inputs of the EEG apparatus were switched off and recording was started only at the termination of it. The switching-on caused a considerable capacitive artifact, the electric seizure, however subsequent to it, appeared overtly in the record. The electric seizure elicited by a 5 sec stimulation consisted of very fast oscillations of low amplitude, lasting for 15—40 seconds. Its type and duration showed only insignificant variations in the same experiment. Immediately after the seizure the base line was flat, spontaneous activity recovered gradually in 2—3 minutes. Electric seizures could be provoked in every 3—5 minutes with relatively constant duration and electrographic pattern.

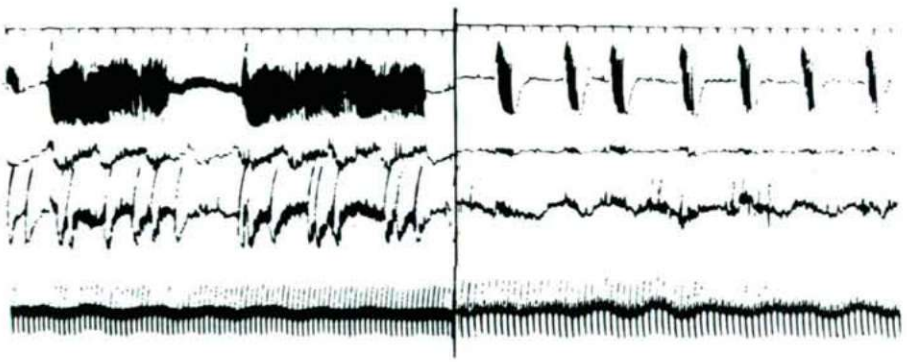


Fig. 6. Rhythmic after-discharge. 1.0 mg/kg N 947. 7 minutes after injection.

In testing tertiary tropin derivatives on the electric seizures we proceeded in the following way. Initially we provoked 3—4 electric seizures with 5 min intervals of rest. Thus the duration and pattern of the seizures characteristic for the animal, was determined. After the last control seizure one of the drugs was injected intravenously in a dose indicated in the respective Figures. (On an animal only one drug was tested.) Afterwards test seizures were elicited in every 5 minutes until the initial seizure pattern recovered. In most animals repeated doses were tested.

In this series of experiments the potency of tropine derivatives was compared with that of the atropine and of several other drugs being in therapeutic use.

Figures 7—10 illustrate the effect of several tertiary tropine derivatives, of atropine and of a commonly used antiepileptic drug.

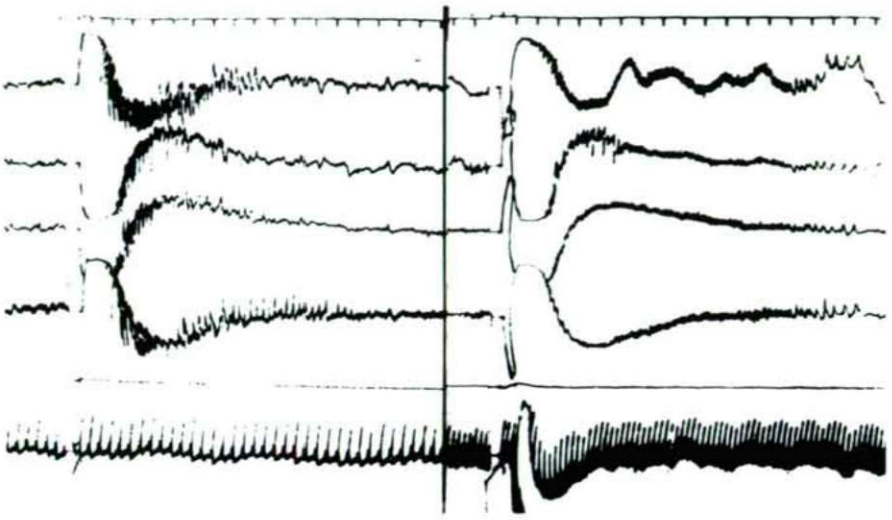


Fig. 7. Cortical electric seizure. At left: control; at right: 7 minutes after injection of 10 mg-kg NA 184.

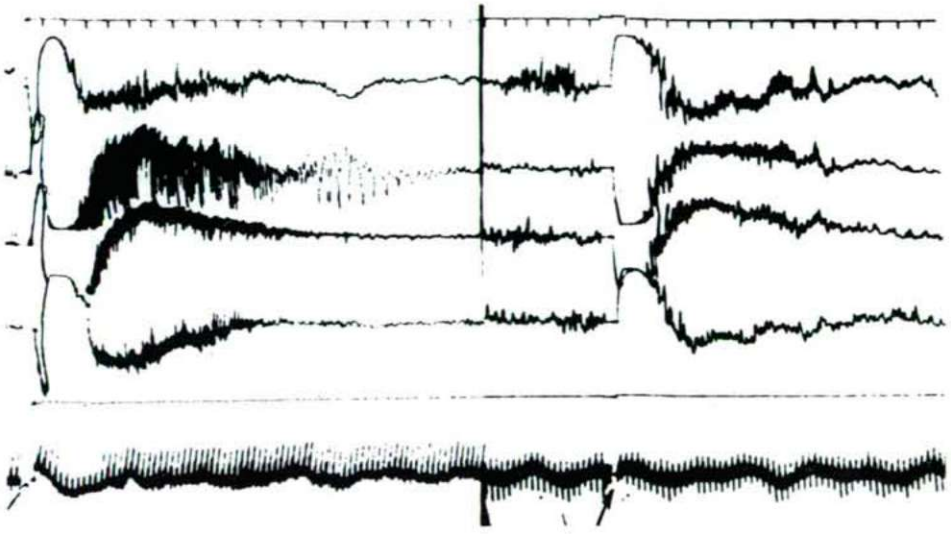


Fig. 8. Cortical electric seizure. 4 mg/kg NA 676; 7 minutes after injection.

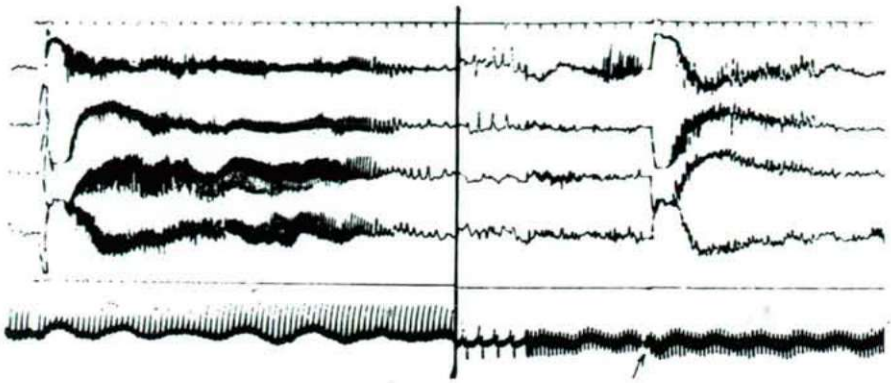


Fig. 9. Cortical electric seizure; 2×1 mg-kg N 947 8 minutes after injection.

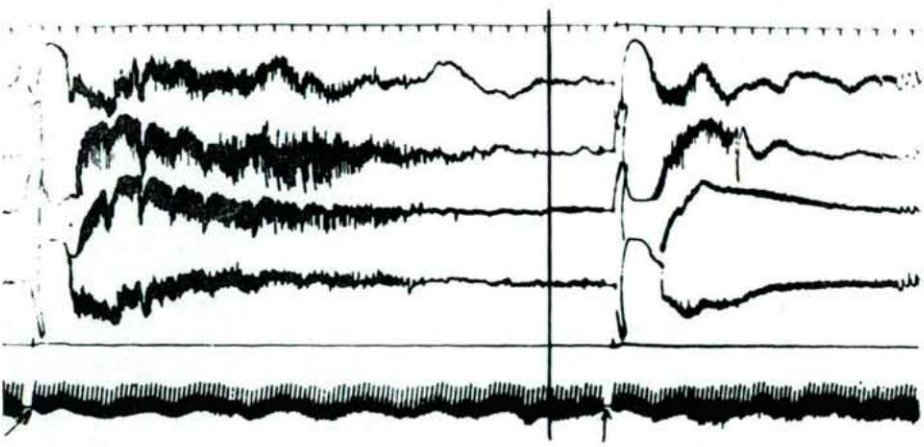


Fig. 10. Cortical electric seizure. 10 mg-kg Diphedan 10 minutes after injection.

**The effect of tertiary tropine derivatives on the electroshock of waking rats**

By means of the procedure described in "Methods" we provoked electroshock on waking rats. After having determined the seizure threshold, a group of rats was injected with a 10 mg/kg dose of a substance to be tested. Test shocks were givent the end of the 1-st, 4-th, and 24-th hour. In several cases the action could be traced up to the eighthday. The small groups did not allow an exact quantitative evaluation, therefore our data obtained from these experiments give only approximate information about this aspect of the anticonvulsive potency. The time courses and estimated values of the anticonvulsive actions are summarized in Fig. 11.

In the experiments reported here, twenty compounds were examined by each testing procedure. Six of them proved to have any anticonvulsive action. The other substances were either toxic or whitout effect.

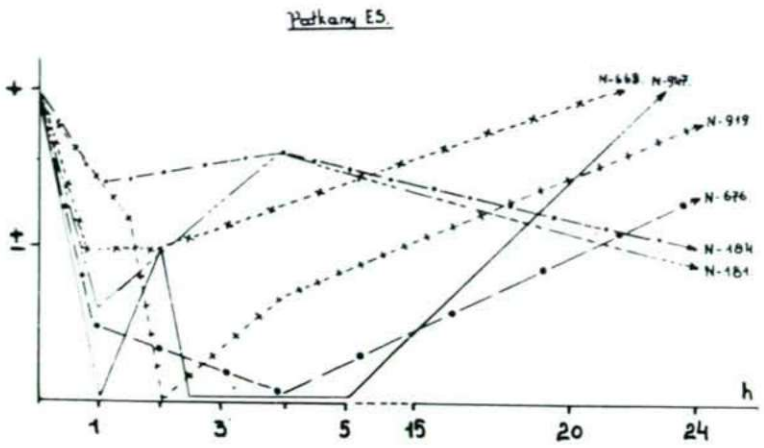



Fig. 11. Approximative anticonvulsive potency of tertiary tropine derivatives as measured on electroshock of waking rats. Ordinate: intensity of seizures; (+) denotes full size electroshock (±) denotes partial electroshock. Points falling on the abscissa denote seizures abolished completely.

## Discussion

Before discussing the anticonvulsive effects of tropine derivatives in detail it should be emphasized, that an exact and all round pharmacological analysis of these compounds fell beyond the scope of our experiments. The main purpose of our work was to control the conclusions drawn from earlier experiments and to accumulate a certain amount of qualitative information about this type of compounds in order to make out if they were suitable for further examination as potential therapeutics.

One of the most obvious results of our investigations seems to be, that the compounds tested were much more powerful especially against the acetylcholine induced after-discharge than the drugs commonly used. In case of the electric seizure of the anaesthetized cat the difference was not as expressed. This may have its origin in the mechanism of the acetylcholine induced convulsions. The tropine derivatives may retain some anticholinergic effect of the atropine and thus they may be capable to antagonize cholinergic processes most powerfully. In the electroshock a lot of other than cholinergic mechanisms may be involved. Despite this fact we assume of high importance that six of the compounds tested, proved to be a potent antagonist of electroshock. This latter is commonly accounted a near analogon of human epilepsy.

As to the correlation between the chemical structure and pharmacological action one can propose only assumptions. It leaves no doubt, that the tropine moiety and especially its tertiary nitrogen plays in the formation of the drug-receptor complex, an outstanding role. The chemical nature of the substituent at the tertiary nitrogen seems to be also highly important. By changing the methyl group for an ethyl radical, as it happens in case of NA 184 and NA 676 respectively, an elevation in potency and duration of the drug action could be attined. A further lengthening of the carbon chain of the substituent e.g. by a propyl group is not advantageous.

Also the acidic moiety represents an important constituent of the molecule. Although it is likely to assume that the attachement to the tropine does not leave intact the original pharmacological properties of the acidic moiety, no doubt remains, that the latters will contribute to the overall pattern of the action of the compound molecule.

The phenyl- and diphenyl-acetic acid is a common component of more substances examined (NA 181, NA 184, NA 676).

According to HANSON (1958, 1959) phenyl-acetic acid inhibits glutamic-acid decarboxylase in brain homogenates and in this way reduces the rate of GABA formation. At 100 μM/3,05 ml drug concentration the inhibition may amount to 96 percent. The exact role of the GABA-shunt in the metabolism is not clear, but it is possible that by inhibition of GABA formation the seizure susceptibility may be elevated.

At the same time a depression of the turnover of glutamic acid may impede the overall cell metabolism and thus reducing excitability.

Another, also not easily evaluable set of data comes from FELLMANN (1956) who observed that phenyl-acetic acid inhibits adrenalin synthesis by depressing DOPA-decarboxylase. Although adrenergic mechanisms are not likely to participate in cortical convulsive phenomena, their role in mediation of subcortical influences cannot be excluded.

GARRATTINI and coll. (1958) threw light on the problem form a different point of view. They could show, that both phenyl- and diphenyl-acetic acid inhibit the synthesis of acetylcholine by preventing the acetylation of coenzyme-A. This may be of immediate importance for cholinergic mechanisms. According to FRUENTOW's report, (1963) diphenyl-acetic acid exerted an inhibitory action on serum cholinesterase. LISSUNKIN (1964) observed that both phenyl- and diphenyl-acetic acid combined with vanadyl-sulphate caused to decrease the acetylcholine content of the brain without enhancing cholinesterase activity. Dann and SUCKER (1964) report about diphenyl-acetic acid and xanthen-9-carbonic acid to have a spasmolytic effect, in which, in the autors' opinion, the inhibitory action upon acetate activating enzymes, plays a definite role. In accord with the data cited previously SZADOVSKA and coll (1964) found not only spasmolytic but also hypotensive effects of esters of diphenyl-acetic acid. These compounds inhibit histamine-contracture of the guinea pig ileum and some of them have analgesic action, too. MEDAKOVIĆ and BANIČ (1963) reported about potentiating effects exerted by some derivatives of the phenyl-acetic acid (CFT 1201 and 1208) on the analgesic action of morphine.

DOYLE and coll. (1965) described expectorant, anti-cholinergic and narcosis-potentiating effects of alcoxymethyl and thiol esters of diphenyl-acetic acid.

A more general picture was given about the effects of phenyl-acetic acid by HICKS and coll (1964) who stated that it inhibited the respiration of the brain tissue, and a wide variety of enzymes, (monoamino-oxydase, glutamic acid decarboxylase, dioxyphenylalanine- and 5-hydroxytryptophane decarboxylase, lactic acid dehydrogenase, glutamic acid- oxalacetic acid- and pyruvic acid- transaminase).

As it is evident from this review far from completeness, phenyl- and diphenyl acetic may influence cellular metabolism in a variety of ways and most of these point to a suppression of cellular activity. The question, however, remains uncleared whether their combination with tropine results in any modifications of the original

pharmacological effects. The tropine itself is assumed to combine with membrane receptors. Its esters behave very likely in analogous manner. This may alter profoundly the pharmacological properties of the acidic moiety. The penetration of tropine esters through the cell membrane awaits to be claered up.

Essentially the same can be told of xanthen-9-carbonic acid and its derivatives. This compound is a component of the substance labelled N 947. The spasmolytic, anticholinergic and antihistaminic effects of these compounds have been shown by several authors (GOLDBERG and WRAGG, 1957, 1960; LIEBER, 1957). We assume that these potencies contribute to the remarkable anticonvulsive action of N 947.

In case of N 919 it is not easy to decide at what extent the acidic moiety plays a role in the final action. The cyclopropane was a widely used anaesthetic (WORINGER and coll, 1951; HAID, 1953; RIKER and coll, 1959) having a presumably analogous mechanism of action with that of other inhalation anaesthetics. It is hardly conceivable that an action being physicochemical in nature could be preserved in that of a compound molecule. About the pharmacological effects of the cyclopropyl-carbonic acid no data are available.

Summing up the results and conclusions presented above it can be stated that there exist remarkable correlations between the chemical structure and pharmacological actions of the tertiary tropine derivatives examined by us. Our experiences may yield fruitful ideas for the synthesis of new potent drugs of this type.

Our conclusions may be summarized as follows.

1. The attachment of the molecule to the appropriate receptors of the neural substrate is made possible by the N-methyl group of the tropine.

2. The duration of the effect is lengthened by N-ethyl substitution. This seems to confirm the assumption included in issue 1.

3. Only tertiary derivatives can be taken into account as anticonvulsive drugs because thay have a chance to penetrate the blood brain barriere.

4. Making a choice of the acidic moiety one has to prefer the ones having similar effects to those wanted from the tropine ester.

5. As tropine esters are not likely to enter the cell they do not interfere with metabolic processes. Therefore chronic toxic effects (autoagressive diseases, injuries of the liver and bone marrow) do not represent a real danger as they do in case of the antiepileptic drugs being in use.

As to the mechanism of action only hypotheses can be put forward. The assumption, however, seems to be justified, that most of them exerts its action by virtue of its anticholinergic potency. Our knowledge about the role of cholinergic synapses in convulsive phenomena is very scarce. The only that can be assumed, is, that a blockage of the depolarizing effect of acetylcholine may be the critical moment at which tropine derivatives interfere with seizure activity. This may be realized by preventing depolarization block of inhibitory neurones and by depressing the overall excitability of cortical cells either. The latter assumption seems to be invalidated by the fact, that tropine derivatives are against strychnine potentials or tetracor seizure without effect. Their excellent protective action against electroshock, however, on anaesthetized cats and waking rats, gives some hope that this type of compounds, may be axpected synthetized, and will contribute effectively to the strugle against human epilepsy.

## Literature


DANN, O., SUCKER H. (1962): Spasmolytisch wirksame Ester und deren Säuren als Hemmstoffe azetat-aktivierender Enzymen. — Arch. Pharmaz. Ber. dtsch. pharmaz. Ges. *295-65*, 497—504.

DECSI, L. (1966): Vizsgálatok az idegrendszerre ható szerekkel. Kandidátusi értekezés.

DOYLE, F. P., MEHTA, M. D., WARD, R., BAINBRIDGE, J., BROWN, D. M. (1965): Chemie und Pharmacologie einiger Ester von basischen Alkoholen. — J. med. Chem. *8*, 571— 76.

FEHÉR, O., HALÁSZ, P., MECHLER, F. (1965): The mechanism of origin of cortical convulsive potentials. Electroencephal. — Neurophys. *19*, 541—548.

FELLMANN, J. H. (1965): Die Hemmung von DOPA-Decarboxylase durch in Verbindung mit Phenylbrenztraubensäureoligophrenie auftretende aromatische Säuren. — Proc. Soc. exp. Biol. Med. *93*, 413—414.

FRUENTOW, N. K. (1963): Bedeutung der Kationengruppe und ihrer Stellung im Molekül für die Reaktion des Substrates un der reversiblen Inhibitoren mit der Cholinesterase. — Biochimija *28*, 964.969.

GARATTINI, S., MORPURGO, M., PASSERINI, N. (1958): Eine Reihe neuer Verbindungen, welche die Cholinacetylierung hemmen. — Experientia (Basel). *14*, 89—91.

GOLDBERG, A., WRAGG, A. H. (1957): Vom Xanthen abgeleitete Spasmolytica. — J. Amer. chem Soc. — *79*, 4226—4229 5/8.

GOLDBERG, A., WRAGG, A. H. (1960): Vom Xanthen abgeleitete Spasmolytica. — J. chem. Soc. (London) 543—455.

GYERMEK, L. (1957): Toxicitási vizsgálatok. — Kísérletes Orvostud. Vizsg. Módsz. *3*, 854—881.

GYÖRGY, L., DÓDA M., NÁDOR K. (1961): Die Bedeutung quaterner Gruppen verschiedener Raumerfüllung für den parasympathicolytischen und den Ganglien-blockierenden Effekt der Tropine. Arzneim. — Forsch. (Drug. Res.) *11*, 444—448.

HAID, B. (1953): Die Cyclopropannarkose. Anaesthesist. — *2*, 128—131 Ang.

HANSON, A. (1959): Die Wirkung von Phenylalaninmetaboliten auf Glutaminsäure-Transaminase in Hirn. — Acta chem. Scand. *13*, 1366—1374.

HANSON, A. (1958): Hemmung der Hirn-Glutaminsäuredecarboxylase durch Phenylalaninmetaboliten. — Naturwissenschaften *45*, 423.

HICKS, J. M., YOUNG, D. S., WOTTON, I. D. (1964): Die Wirkung uremischer Blutkonstituenten auf bestimmte Hirnenzyme.[1]— Clin. chim. Acta (Amsterdam) *9*, 228—235.

LIEBER, C. S. (1957): Vergleichende Untersuchung über die hemmende Wirkung des Atropins und zweier synthetischer Parasympathicolytica auf die Magensaftsekretion. — Ann. Soc. roy. Sci. med. natur. (Bruxelles) *10*, 209—321.

LISSUNKIN, J.n I. (1964): Der Acetylcholin-Stoffwechsel des Rattenhirns bei kombinierter Einwirkung von Vanadylsulfat und einigen Phenylessigsäurederivativen. — Bull. Exp. Biol. Med. (UdSSR) *57*, (29.) 2, 80—83.

MEDAKOVIC, M., BANIC, B. (1963): Der Einfluss von zwei Phenylessigsäurederivaten auf die analgetische Wirkung von Morphin in Mäusen. — J. Pharmacy Pharmacol. *15*, 660—665.

NÁDOR, K. (1958): A gyógyszerkutatás elvi problémái. — MTA Biol. Oszt. Közleményei. *8*, 187—216.

RIKER, W. F., WERNER, G., ROBERTS, J., KUPERMAN, A. (1959): Pharmacologischer Beweis für die Existenz eines präsynaptischen Vorgang bei der neuromusculären. Übertragung. — J. Pharmacol. exp. Ther. *125*, 150—158.

WORINGER, E., BROGLY, G., SCHNEIDER, J. (1951): Untersuchung der Wirkung allgemein gebräuchlicher Anästhetica auf den Druck der Rückenmarksflüssigkeit. Praktische Bemerkungen auf ihre Verwendung in der Nervenchirurgie. — Anesthésie et Analgésie *8*, 649—662.



Address of the authors:
Prof. Dr. O. FEHÉR
G. A. BÁLINT
Á. BALOGH
Department of Animal Physiology
A. J. University,
H—6701 Szeged, P. O. Box 428.,
Hungary

Acta Biologica Szeged. *21* (1—4), pp. 127—140 (1975)

# THE EFFECT OF POLARIZATION ON THE EVOKED AND SEIZURE POTENTIALS IN THE CAT

M. SZENTE and O. FEHÉR

*Department of Animal Physiology, Attila József University, Szeged*

(Received March 25, 1975)

**Abstract**

The effects of polarization was studied on cortical auditory evoked and strychnine potentials. Hyperpolarization augmented the acoustic evoked potentials in deep leads, depolarization depressed them. Polarization resulted on strychnine potentials in changes of proportions of different phases without altering the peak-to-peak amplitude. The authors assume, that modifications in transmitter output from presynaptic endings may play a considerable role in the mechanism of polarizational effects.

The mechanism of interneuronal transmission of impulses at the chemical synapses is in many aspects uncleared, up to now. This problem has aroused an intensive interest among morphologists, biochemists, and even physicists, too.

The interneuronal transmission as a whole can be divided into four distinct moments as follows: i) invasion of the presynaptic ending by the propagating axonal spike, ii) the liberation of transmitter substance from its place of storage, iii), the diffusion of the transmitter through the synaptic gap to the postsynaptic membrane, if condunctance changes of the postsynaptic membrane.

The aim of our work was to study the finer details of these processes, especially those taking place on the presynaptic side in cortical synapses.

The experiments were performed on the acoustic cortex of the cat. Primary evoked potentials were elicited by application of click-stimuli to one ear. The frequency dependence of the evoked potentials and the effect of electric polarization was studied. On the basis of the data obtained in this way we draw conclusion as to the quantitative aspects of transmitter dynamics in cortical synapses.

## Methods

The experiments were performed on adult cats of 2,5—3,0 kg weight, anaesthetized by 40 mg/kg pentobarbital-Na given intraperitoneally. The femoral vein and trachea were cannulated. For maintaining the level of anaesthesia 5—10 mg/kg pentobarbital-Na was given additionally.

The head of the animal was fixed in a stereotaxic instrument (type-Kovách), the skin and the muscles of the scalp were removed bilaterally. By use of a dental drill we opened the skull and exposed the gyri ectosylvius and suprasylvius on both sides. The dura was cut with fine scissors. Warm paraffin oil protected the brain surface from cooling and drying. Bone-bleeding was prevented by wax. After surgery the animal remained in rest for 1—2 hours. Body temperature was kept constant by an electric heater.

Recording.

Acoustically evoked potentials were led off from the primary auditory area, from a depth of 1500—2000 micra. The recorded site was always at the *punctum maximum* of these potentials. As a

recording electrode we used a steel-needle of 0,3 mm in diameter, isolated up to the tip. The electrode, fixed to the holder of the stereotaxic instrument could be moved in three dimensions. Depth measurements were possible with 0,05 mm accuracy. As the introduction of the electrode inevitably compressed the cortex and falsified depth measurement, the electrode was introduced somewhat deeper and the depth wanted was attained during withdrawal. The tip of the electrode remained at the depth where deep negative evoked potentials appeared to be maximal. After having inserted the electrode at the appropriate depth, 15—20 minutes rest was given for restitution of the cortical circulation. A silver wire was attached to the skin of the head as an indifferent electrode. The signals to be amplified were led into a DISA two-channel Electromyograph with a time constant of 120 msec. For data storage we used a magnetic tape recorder.

*Stimulation.* For evoking primary acoustic potentials we used click stimuli through an earphone applied to the right ear. Clicks were produced by 35 V, 1 msec impulses of a square-wawe stimulator. Stimulation was always supramaximal.

*Polarization.* We applied polarizing electrodes on the anterior ectosylvian gyri on both sides. In this way the polarization was transcortical. The polarizing circuit included a battery, a microammeter, a potentiometer and a polarity-switch. As polarizing electrodes we used chlorided silver plates of 0,6 cm surface area. Thus the current loops traversed the cortex, the underlying white matter and the commissural fiber system (Fig. 6).

The intensity of the polarizing current was adjusted to cause a voltage-gradient of 30 mV/mm extracellularly. This voltage-gradient could be maintained in most cases by a current of about 400 $\mu$A. The contact of the polarizing electrode to the cortex did not disturb the cortical circulation. On the polarizing electrode of the left side there were small holes for the recording electrode. When the positive electrode was on the recorded side, we regarded it as an anodal or hyperpolarization. The polarization started 1 minute before stimulation and acted till the end of it. After each polarization period 5 minutes rest followed for restitution from polarizational changes.

*Evaluation of data*

The storage of the signals on magnetic tape enabled us to make amplitude averaging. This was done by means of the 512 channel amplitude analyser (KFKI typ. NTA 512) placed kindly at our disposal by the Dept. of Physiology of the Medical School in Szeged. At every stimulation frequency 100 signals were averaged. Channel width was 128 msec at frequencies 1—5, analysis time 0,5 sec. At 7— to 10 cps channel width was 64 msec. An X—Y recorder fed from the amplitude averager delineated the experimental curves.

## The effect of DC polarization on the evoked potentials

The polarizing current, whose loops extended between the ectosylvian gyri of the two sides, resulted in a voltage change of 30 mV/mm at 400 $\mu$A current strength. The effect of polarization on the frequency dependence curves can be seen in Fig. 1. The diagrams include the results of six experiments. As it is apparent in this Figure, hyperpolarization shifts the whole frequency dependence curve toward higher amplitudes; depolarization has the opposite effect. In the first two experiments depolarization causes the appearance of a new inflexion point; this may be the consequence of a shift of the upper inflexion point toward higher frequencies.

In course of the evaluation special attention was paid to the question: how the polarization induced changes of the frequency dependence curves could be fitted to the curves computed on basis of our theory about transmitter dynamics (FEHÉR, HUNYA, 1973). A further question was: which parameters of the model could be made responsible for changes observed during polarization.

The block diagram clearly demonstrates, that the proportion of transmitter liberated by one iinpulse was decreased by depolarization and increased by hyperpolarization. The extent of these changes could be estimated quite accurately by analysing the frequency-dependence curves. This shows a good agreement with

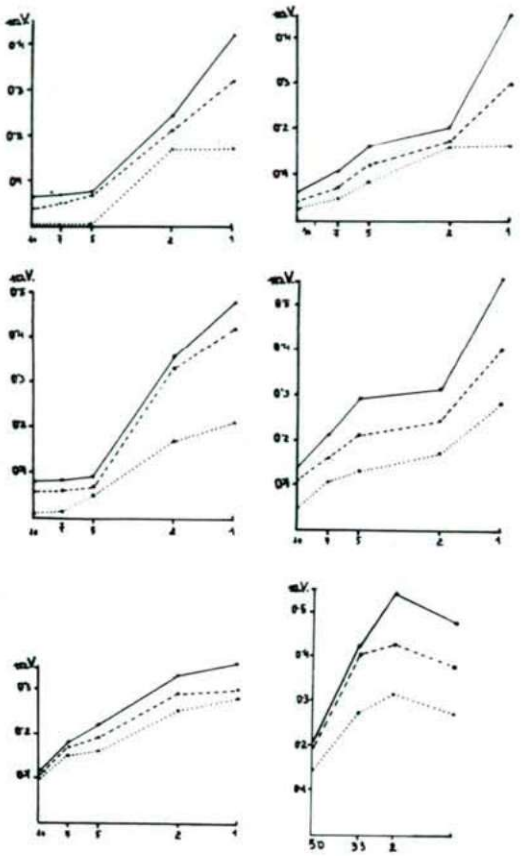


*Fig. 1.* Effect of polarization on the amplitude of evoked potentials. The diagrams represent the results of six experiments. Ordinates: amplitudes in μV. Abscisses: stimulation frequencies on logarithmic scale. Broken lines: control series, continuous lines: frequency dependences curves taken during hyperpolarization, dashed lines: the same during depolarization. Polarizing current intensity was 400 μA in each case.

conclusions drawn from experiments on muscles; according to these, the amplitude of the spike potential is the main factor determining the liberated transmitter quantities from synaptic endings. One parameter of our model (FEHÉR, HUNYA, 1972) reflects the proportion of transmitter liberated by one impulse quite accurately and therefore conclusions can be drawn from changes of frequency dependence curves as to the mechanism of action of any factor acting upon transmitter dynamics. Unfortunately it is not easy to judge which one of the factors can be made responsible for changes in transmitter proportions liberated by one impulse: the duration of action potentials or modification of permeability of the presynaptic endings. Presumably, the form er more constant and thus permeability changes lead more likely to changes in transmitter output.

As it was mentioned above, in experiments with polarization the upper inflexion point on the frequency dependence curve could not be found. Its situation on the curve is influenced by transmitter synthesis. No data are, however, available so far according to which polarization would exert any effect upon this process. Thus, the lack of the upper inflexion point does not curtail the usefulness of our model.

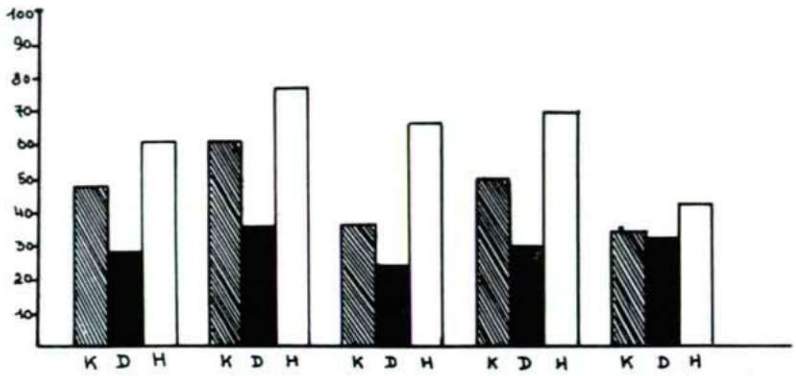



*Fig. 2.* The evaluation of five experiments illustrated in Fig. 1. Ordinate: The percentual proportion of the transmitter store, liberated by one impulse. Designations of blocks: K: control, D: Depolarization, H: hyperpolarization.

### The effect of polarization on strychnine potentials

Strychnine potentials were evoked by applying 1 percent strychnine solution on the cortex by a 2×2 mm filter paper soaked in it. As the site of origin of strychnine potentials lies in the superficial layers, we examined the effects of polarization in surface leads. The strychnine potentials follow stimulation frequencies over 3 cps rather poorly. Therefore, their frequency dependence could not be examined. The lack of these curves as to the strychnine potentials did not prove to be disadvantageous because polarization failed to exert any effect on them at 1 and 2 cps in surface leads. This is illustrated in Fig. 3 by synchronized records in control situation, at hyper- and depolarization, respectively.

Our findings in deep leads were seemingly contrary (Fig. 4). The strychnine potentials exhibiting a negative-positive-negative sequence in depth records, underwent characteristic changes during polarization; depolarization enhanced their negative phase while the positive wave decreased or disappeared completely. Hyperpolarization, on the contrary, depressed the negative phase and enhanced the deep positive waves. The peak-to-peak amplitude, remained, however, unchanged. The effect of polarizing current consisted in a shift of the DC-level and the strychnine potentials appearing on different background potential level showed variations only in proportions of their positive and negative phases. Depolarization emphasized their negative, hyperpolarization augmented their positive components.

A similar phenomenon could be observed also at the evoked potentials, but it was joint always with changes of the peak-to-peak amplitude described above.

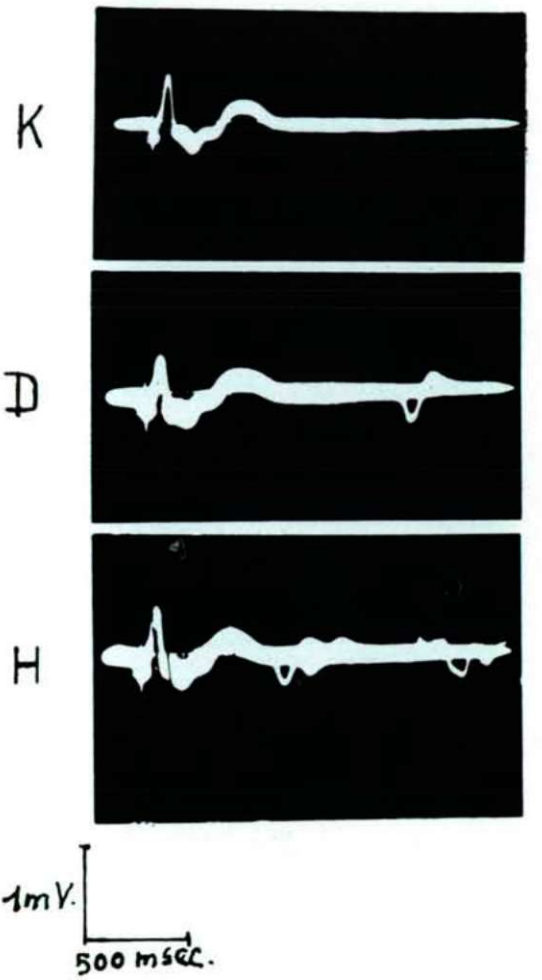



*Fig. 3.* Effect of polarization on strychnine potentials in surface leads. Ten superimposed traces photographed from the screen of an oscilloscope. Negativity upwards. Calibration: 1 mV and 10 msec. K: control, H: hyperpolarization, D: depolarization.

Analysing the effects of the polarizing current on cortical structures, the first question, that arises concerns their explicability in terms of the electrotone observed on peripheral nerves. As it is well known, the membrane potential of peripheral nerves decreases in catelectrotone with a concomitant increase in excitability and conductivity. Anelectrotone causes changes of opposite sign.

No doubt, the cortical polarization phenomena show remarkable similarities to those observed in peripheral electrotone. The conditions under which the polarizing current acts are, however, different in many respects in the two structures. i) The polarizing current traversing the cortical tissue makes its way through the intercellular

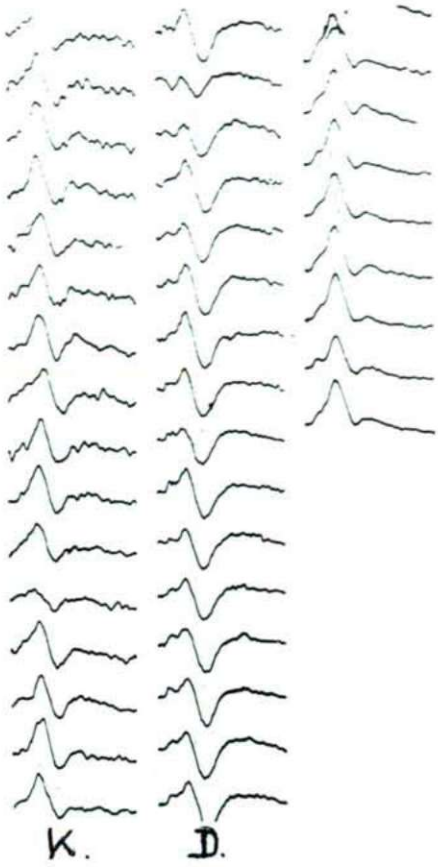



*Fig. 4.* Effect of polarization on strychnine potentials lead off from a depth of 1500 μ. K: control, D: depolarization, H: hyperpolarization. Although peak-to-peak values do not vary significantly, deep negative phase is augmented during hyperpolarization, and deep positive phase changes in the same direction during depolarization.

space and thus the current intensity entering the neural elements is rather low. ii) In the cerebral cortex one must reckon not only with effects on conductivity but also with those directed to transmission of impulses, iii) In peripheral nerves propagated spikes of "all, or nothing" type serve as indicators for polarizational effects; in the cerebral cortex evoked potentials, composed of PSP-s were subjected to examination.

In the following discussion we make an attempt to explain the mechanism of the polarizational changes and to answer the question: to what extent they can be attributed to shifts of membrane potential and to what extent one has to take into account actions exerted on interneuronal transmission.

Our analysis starts from the electric membrane model constructed on the basis of the cable theory and described in detail by COLE (1968).

The physical system modelling the nerve membrane is illustrated in Figure 5. In this $R_a$ represents the resistance of the external medium of unit length (200 Ohm cm); $R_i$ is the resistance of the internal medium of unit length (60 Ohm cm); $R_m$ is the resistance of the membrane of unit area (2000 Ohm cm²); $I_a$ represents the current flowing in the external medium, $I_i$: the current flowing within the nerve fiber; $I_m$ is the membrane current. (The electric parameters were taken from Aidley:

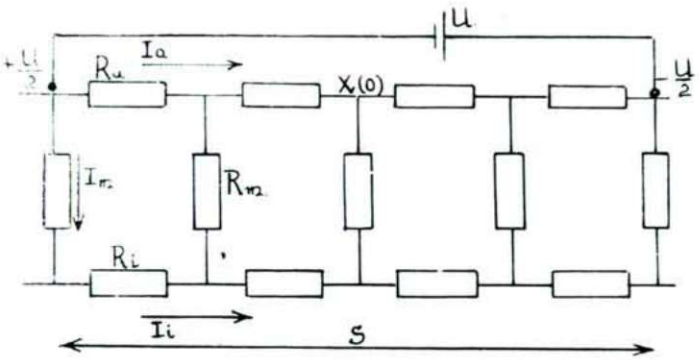



*Fig. 5.* The electric model of nerve cell membrane, simplified for stationary DC relations. Explanations see in the text.

"The physiology of excitable cells".) The equations describing the respective voltages appearing on the outer and inner membrane surfaces are according to Beier:

$$(1) \qquad U_a = \frac{U}{K + \coth\frac{s}{2\lambda} + 2\frac{\lambda}{s}\frac{R_a}{R_i}} \cdot \left[ -\frac{\lambda}{s} \cdot \frac{R_a}{R_i} \cdot \frac{\sinh\frac{x}{\lambda}}{\sinh\frac{s}{2\lambda}} - \left(K + \coth\frac{s}{2\lambda}\right)\frac{x}{s} \right]$$

$$(2) \qquad U_i = \frac{U}{K + \coth\frac{s}{2\lambda} + 2\frac{\lambda}{s}\frac{R_a}{R_i}} \cdot \left[ \frac{\lambda}{s} \frac{\sin\frac{x}{\lambda}}{\sin\frac{s}{2\lambda}} - \left(K + \coth\frac{s}{2\lambda}\right)\frac{x}{s} \right]$$

The difference $U_a$—$U_i$ represents the membrane potential change during polarization from a battery of voltage U. K is a constant depending on the shape of the electrodes. $\lambda$ is the length constant and equals, $\lambda = \sqrt{\frac{R_m}{R_a + R_i}}$ · x is the distance of the nerve portion under examination from the middle of the intrapolar zone. The validity of the equations is restricted to stationary DC potentials.

The physical relations described above are realized among the special conditions of the cat's cerebral cortex as follows (Fig. 6a).

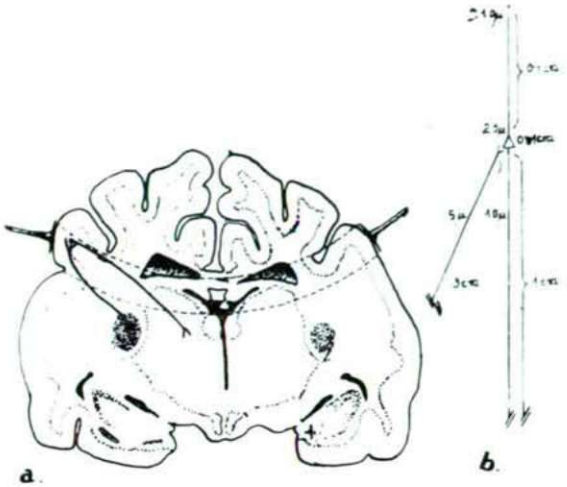


*Fig. 6a.* The anatomical circumstances of polarization. (Explanation see in the text.)
*Fig. 6b.* An idealized pyramidal cell with thalamocortical afferent fibers. Numbers on the right refer to length, those on the left refer to diameters of fibers. These sizes served as basis for calculations of changes in membrane pontetial.

The two polarizing electrodes were situated on the ectosylvian gyri of both sides. Their shortest distance was 6 cm, from each other and 3 cm from the medial geniculate body of the same side. The geniculocortical pathway fell presumably at least in 1 cm length into the path of the polarizing current.

As to the geometric parameters of the vertically oriented cells (pyramidal neurones of different layers) the following assumptions were made (Fig. 6b).

The hypothetic pyramidal neurone is situated in 1 mm depth; its apical dendrite reaches the cortical surface and makes an arborization parallel with it. The radius of the dendrite is 3 μ at its origin while at the final branches 0,1 μ. The thalamocortical axon runs about 1 cm parallel with the current loops. Its radius diminutes gradually from 5 μ to 0,1 μ at the terminal branching. Its terminal arborization was supposed at the final portion 100 μ in length. The soma was regarded as a cone of 100 μ height with a lower diameter of 30 μ and an upper one of 6 μ. The longitudinal axis of the soma was assumed to be parallel with the direction of current.

By making use of the equations (1) and (2) at different sites of the pyramidal cell and at the thalamocortical endings, the following membrane potential changes were calculated at an extracellular potential gradient of 30 mV/mm.

at terminal branching of apical dendrites $\pm 6{,}7$ mV;
at terminal branching of thalamic afferents $\pm 16{,}0$ mV;
at the soma of pyramidal neurones $\pm 20{,}0$ mV.

The bias of membrane potential changes listed above is negative at depolarizing and positive at hyperpolarizing currents. Because of the complicated geometry of the basal dendrites, the assumptions made for the soma region could only be approximate. As it turned out of the calculations, the polarization changes depended mainly on the length of the nerve portion parallel to the current flow, but the diameter of the nerve fibres plays a considerable role as well.

Considering the main results of our experiments several observations deserve special attention and discussion.

It was noteworthy that strychnine potentials did not undergo in surface leads any change during polarization as regards their amplitude, or in proportions of their component waves, either. In deep leads, however, hyperpolarization enhanced the deep positive and depolarization the deep negative waves, without altering the peak-to-peak amplitude. These seemingly contrary findings might have been resulted by different conditions for electric conduction.

In the neuropile of the superficial layers consisting of very fine dendritic branching one has to reckon with the high internal resistance of the axoplasm. Therefore the polarizing current intensity and the voltage gradient across the membrane remained relatively low. In an apical dendrite 1 mm length of which falls in the direction of the polarizing current, the polarization voltage gradient was calculated to be about 6,7 mV. Its effect on the amplitude and phase relations of the surface phenomena could only be moderate.

The obvious changes of phase relations in deep leads resulting from polarizational effects may be explained by the modifications of the membrane potential in the soma and basal dendrites. The hyperpolarization of the soma and the basal dendrites will augment the EPSP-s, while depolarization should act in the opposite direction. This seems to be valid also for hyperpolarizations arising from real surface depolarizations by means of currents carried by the volume conductor of the cortex. The surface negative spike of the strychnine potential is corresponded by a deep positive wave; this latter is suppressed by hyperpolarization, because of the elevation of the membrane potential.

The question may now be raised, whether polarizational effects on thalamocortical fibers acted upon liberation of transmitters as it was shown by ECCLES and coll. (1962) in spinal cord, by HUBBARD (1962a, b) at the neuromuscular junction. Any significant change in amplitude of strychnine potentials could not be seen during polarization. In explaining this, we assume, that the enormous irradiation of excitation represented by the strychnine potentials takes its origin from a widespread excitation of the superficial dendritic mass and a great number of short-axon interneurones which are influenced relatively weakly by the polarizing current. Based on equations (1) and (2) one can calculate, that a polarizing current of 400 μA causes a membrane potential change of only 0,5 mV in an axon, having a diameter of 5 μ

The polarizational changes of the evoked potentials are of quite different origin. Their most significant feature is a change in peak-to-peak amplitude. Hyperpolarization causes an increase in amplitude without altering the proportions of the negative and positive phases. Depolarization acts in the opposite sense. These modifications may find their explanation in variations of transmitter release from the thalamocortical endings. This latter assumption was based on the analysis of the frequency dependence curves obtained experimentally and by means of computer calculations. According to equations (1) and (2) a gemuculocortical afferent fiber suffers a membrane potential change of 16,0 mV, if it has a portion of at least 1 cm in length prallel with the polarizing current. Assuming that membrane potential changes influence the transmitter output at about the same extent as they do at peripheral terminals, a hyperpolarization of 16,0 mV may be able to treble transmitter release. Presumably, depolarization is as effective in the opposite direction. As

measurements concerning the effect of polarizing current upon transmitter release in the cortex are lacking, we quote data of HUBBARD (1962a), about the correlation between the amplitude of potsynaptic EPSP-s and the membrane potential of he-presynaptic fibres causing them to appear. The change in transmitter output ctal culated by use of the model finds its explanation, at least qualitatytively, in the presumed modifications of the presynaptic membrane potential.

The next question, that may be raised, concerns the lack of changes in the proportion of the negative and positive phase of the evoked potentials despite the considerable influence of the polarizing current on the soma and basal dendrites. As it was mentioned earlier, an effect of this type was observed at 1 cps frequency but not at higher ones, producing evoked potentials of smaller amplitude. The reason for this seems to be, that membrane potential changes affect large evoked potentials at a greater extent than the smaller ones. As an example for this, strychnine potentials can be quoted, which represent nearly maximal depolarization of the neuronal membrane.

The fundamental data regarding the effect of DC polarization upon nerve endings have come from experiments on the neuromuscular junction. On a relatively simple frog nerve-muscle preparate del CASTILLO and KATZ (1954) found, that by applying current on the motor axon, considerable changes occurred on the miniature endplate potentials and on those elicited by motor axon stimulation. Cathodal polarization caused an increase in the amplitude of the miniature EPP's, while anodal polarization remained ineffective. In these cases electrotonic changes did not extend to the postsynaptic side. EPP's elicited by motor axon stimulation were augmented during anodal polarization.

The effect of repetitive synaptic stimulation on the EPSP-s of spinal motor neurons was studied by CURTIS and ECCLES (1960). They presumed, that the EPSP amplitudes are proportional to the transmitter action evoking them. In their opinion, a motor volley initiates two processes in the junctional apparatus: i) an enhancement of 200 msec in duration and ii) a subsequent depression which may last for seconds. By applying repetitive stimuli a constant level of EPSP amplitude could be attainel which, usualy, is below the normal one. If the stimulus frequency was between 4 and 20 cps, the depression became more intensive by 0,3 sec after the beginning of stimulation and the EPSP amplitudes decreased to 70—80 percent of the initial value. At higher frequencies a potentiation appeared that reached its maximum between 30 and 100 cps. This points to the fact, that the charge of the transmitter store is not of constant intensity and depends on the stimulation frequency. This is contrary to the findings of ZABLOCKA—ESPLIN (1972). A good correlation was found between the amplitude of the second EPSP in a series and the average amplitude of the same series. This fits well with our experiences. At very high frequencies, the EPSP amplitude decreases progressively and over 250 cps, becomes inversely proportional to the time separating the impulses. This means that the transmitter output had attained its maximal rate. For this and other phenomena, not reported here, four basic mechanisms can be held responsible: i) a partial blockage of the presynaptic volley near to the final axon branchings, ii) hyperpolarization with consecutive augmentation of the spike potential, iii) modifications of the transmitter output from presynaptic endings and iv) changes in sensitivity of the postsynaptic membrane against the transmitter substances. Possibilities listed under i) and iv), could be excluded by appropriate control experiments. Thus all phenomena of facili-

tation and depression could be ascribed to respective changes of the presynaptic spike and transmitter output. The effects of polarization on the EPSP's of rats phrenic diaphragm preparation were studied by HUBBARD and WILLIS (1962). During hyperpolarization the EPSP-amplitudes rose to ninefold, while depolarization resulted in a considerable decrease. In explaining these phenomena they took into account mainly the variations of the transmitter output. In another series of experiments HUBBARD and SCHMIDT (1963) confirming their earlier results, observed that rhythmic stimulation enhanced presynaptic spike amplitude considerably and the heights of the postsynaptic potentials seemed to depend on them in a logarithmic manner. In case of paired stimuli, the second EPP proved to be higher. The mechanism of the potentiating action had to be sought in a different way, because the second presynaptic spike was usually smaller in these cases.

Our specific field of interest is more directly concerned by the extensive literature dealing with modifications of the evoked potentials during the passage of polarizing currents. In this place only data of immediate importance will be cited. BISHOP and O'LEARY (1950) has described essentially the same effects of polarization on the cerebral cortex as we did. In explaining their experimental findings they take into account only postsynaptic processes and neglect presynaptic ones at all. According to them, different phases of the evoked potentials can be deduced from activity of different cell groups. Their interpretation however does not reckon with the effect of the volume conductor of the cerebral tissue. The effects of polarization on cortical responses produced by epicortical stimulation were subjected to investigation by CASPERS (1960). In his experiments anodal polarization enhanced dendritic potentials negative in sign; cathodal current depressed and, later, inverted them. Interestingly, the actions of GABA were counteracted by hyperpolarization, while depolarizing currents proved to be synergistic. Considering that the mode of provoking surface dendritic potentials was quite different from that applied by us, analogies can be drawn only with caution. It seems to be sure, that the current intensities used by CASPERS (1960) were high enough to cause membrane potential changes in the superficial dendritic mass, too.

Basing on earlier works of RUSINOW MORREL (1961) has shown that surface anodal polarization facilitated the discharges of cortical neurones recorded intracellularly by microelectrodes. Another highly important observation was in his experiments, that cortical neurones retained their newly acquisited conditional-connections better, if they stood under the effect of hyperpolarizing currentat the time of the conditioning procedure. On conditional stimuli they exhibited positive responses at times, when other cells failed to respond at all.

HERN and coll. (1962) observed, that the electric threshold of the motor cortex was much lower to anodal stimuli, than to cathodal ones. They assume, that anodal stimuli excite pyramidal cells directly, the cathodal impulses do it only in in tnraspresynaptic way. According to LIPPOLD and coll. (1962) a hyperpolarization applied to the somato-sensory cortex augmented the positive phase of the evoked potential and enhanced the unit discharges coinciding with it. Depolarization acted in the opposite sense. The polarizational after-effects lasted about 20 minutes. DENNEY and BROOKHART (1962) subjected recruiting and augmenting responses to polarizion currents of 250—1000 μA and 200—300 μA intensity. Their leads were made from the surface. Hyperpolarization enhanced mainly the surface negative waves, depolarization depressed them. According to their interpretation, the polarizational changes

mentioned above are due to membrane potential changes of neural elements producing graded electric responses. This view has been confirmed by BINDMAN et ol. (1964) in commenting their studies carried out by means of polarization through microelectrodes.

PURPURA and SHOFER (1964) were able to induce membrane potential changes in cortical units through microelectrodes. They brought clear evidences according to which the height of EPSP's or IPSP's was modified depending on the direction of the membrane potential shift as compared to the respective equilibrium potentials of the two postsynaptic potentials. Their evidences favour the view that evoked potentials represent the sums of local potentials in cortical neurones.

LANDAU and coll. (1964) applied polarizing currents at the cortical surface and at different depths. The modifications of the evoked and strychnine potentials observed by them are essentially the same as those seen in our experiments. However, at variance with our findings they did obtain changes in the phase relations of the surface strychnine potentials. The current intensities, applied by them exceeded those used by us 2—3 fold. As to the effect of polarization upon the presynaptic endings most direct evidences can be derived from the experiments of PURPURA and MCMURTRY (1965). They recorded the membrane potential changes of PT and non-PT-cells of the cat's motor cortex intracellularly ensuing on orthodromic an antidromic stimuli during polarization of both signs. Anodal polarization enhanced the activity of PT-cells, cathodal currents depressed it. The membrane potential changes provoked in this way were not very significant. On non-PT-cells, at variance, surface anodal polarization caused hyperpolarization with corresponding modification of activity. The strong polarizational effects obtained on surface augmenting responses migth be due to the circumstance that their current intensities were several times higher than our ones. According to the experiences of RABINOVICH and KOPYTOWA (1969) neurones of the rabbit's motor cortex retained a long lasting higher reactivity after a period of hyperpolarization while the responsiveness of those which had not been hyperpolarized previously was lost rapidly. Thus, there appears, that hyperpolarization exerts an overt facilitatory effect obn the quasi-conditioned responses of cells stimulated from different sources. Similar positive after-effects were observed by VORONIN and SOLNTZEWA (1969) on the rabbit's sensory-motor cortex after surface positive polarization. This lasted up to 10 seconds without significant alterations in the membrane potential. The main conclusion that can be drawn from their observations is, that the effect of surface positive polarization is directed mainly to the transmitter output of the presynaptic endings.

In comparing our experimental results with the data of the extensive literature referring to our problem, the conclusion seems to be justified, that DC polarization of the cerebral cortex may not only be effective by altering the membrane potential of postsynaptic elements but also through modification of the transmitter release from presynaptic endings. Its seems very likely, that this latter type of action may play an important role both in the establishment of new conditional connections and in preservation of memory traces. The polarization and especially the anodal polarization of the cerebral cortex creates therefore an excellent model situation for the study of fixation of memory traces at the cellular level.

## Literature


BEIER, W. (1962): Biophysik, VEB Georg Thieme, Leipzig.

BINDMAN, L. J., LIPPOLD, O. C. REDFEARN, J. W. T. (1964): Relation between the size and form of potentials evoked by sensory stimulation and the background electrical activity in the cerebral cortex of the rat. —J. Physiol. *171*, 1—25.

BISHOP, G. H., O'LEARY, J. L. (1950): The effects of polarizing currents on cell potentials and their significace in the interpretation of central nervous system activity. — EEG clin. Neurophysiol. *2*, 401—416.

CASPERS, H. (1960): Die Wirkung von Gamma-Amino-Buttersäure und Substanz P auf die bioelektrische Aktivität der Hirnrinde. — Medicina Experimentalis *2*, 198—212.

DEL CASTILLO, J., KATZ, B. (1954): Changes in end-plate activity produced by pre-synaptic polarization. — J. Physiol. *124*, 586—604.

COLE, K. S., LI, C. L., BAK, A. F. (1969): Electrical Analogues for Tissues. — Exp. Neurology **24**, 459—473.

CURTIS, D. R., ECCLES, J. C. (1960): Synaptic action during and after repetitive stimulation. — J. Physiol. *150*, 374—398.

DENNEY, D., BROOKHART, J. M. (1962): The effects of applied polarization on evoked electrocortical waves in the cat. — EEG. clin. Neurophysiol. *14*, 885—897.

ECCLES, J. C., KOSTYUK, P. G., SCHIDT, R. F. (1962) Central pathways responsible for depolarization of primary afferent fibres. — J. Physiol. *161*, 237—257.

FEHÉR, O., HALÁSZ, P., MECHLER, F. (1965): The mechanism of origin of cortical convulsive potentials. — Neurophys. *19*, 541—548.

FEHÉR, O., HUNYA, P. (1973): On the nature of frequency dependence of the evoked potentials in the cat's auditory cortex. — Acta Biochim. et Biophys. Acad. Sci. Hung. *8*, 45—56.

HERN, J. E. C., LANDGREN, S., PHILLIPS, C.b G., PORTER, R. (1962): Selective excitation of corticofugal neurones by surface-anodal stimulation of the baboon's motor cortes. — J. Physiol. *161*, 73—90.

HUBBARD, J. I. (1962a): Hyperpolarization of mammalian motor nerve terminals. — H. Physiol. *163*,

HUBBARD, J. I. (1962b): Reduction of transmitter output by depolarization. — Nature (Lond.) *193*, 1294—1295.

HUBBARD, J., SCHMIDT, R. F. (1962): Repetitive activation of motor nerve endings. — Nature, (Lond.) *196*, 378—379.

LANDAU, W. M., BISHOP, G. H., CLARE, M. H. (1964): Analysis of the form and distribution of evoked cortical potentials under the influence of polarizing currents. — J. Neurophysiol. *27*, 788—813.

LIPPOLD, O. C. J., REDFEARN, J. W. T., WINTON, L. J. (1961): The potential level at the surface of the cerebral cortex of the rat and its relation to the cortical activity evoked by sensory stimulation. — J. Physiol. *157*, 7 P.

MORRELL, F. (1961): Effect of anodal polarization on the firing pattern of single cortical cells. — Ann. N. Y. Acad. Sci. *92*, 860—876.

PURPURA, D. P., MCMURTRY (1965): Intracellular activities and evoked potential changes during polarization of motor cortex. — J. Neurophysiol. *28*, 166—185.

PURPURA, D. P., SHOFER, R. J. (1964): Cortical intracellular potentials during augmenting and recruiting responses. I. Effects of injected hyperpolarizing currents on evoked membrane potential changes. — J. Neurophysiol. *27*, 117—132.

RABINOVICH, M. YA., KOPYTOVA, F. V. (1969): Effects of combining polarization of the cortica motor zone with acoustic stimulation. — ZH Vyssh. Nerv. Deyatel. *19*, 768—777.

RUSINOV, V. S. (1953): An electrophysiological analysis of the connecting function in the cerebral cortex in the presence of a dominant region area. — Abstr. Comm. XIX. Internat. Physiol. Congr. Montreal: 719—720.

VORONIN, L. L., SOLNTZEVA, E. I. (1969): After effects of polarization of single cortical neurons. Intracellular recording. ZH. Vyssh. Nerv. Deyatel. *19*, 823—838.

ZABLOCKA—ESPLIN, B., DON ESPLIN, W. (1971): Persistent changes in transmission in spinal monosynaptic pathway after prolonged tetanization. — J. Neurophysiol. *34*, 860—867.

Address of the authors:
Dr. M. SZENTE
Prof. Dr. O. FEHÉR
Department of Comparative Physiology
A. J. University,
H—6701 Szeged, P. O. Box 428,
Hungary

Acta Biologica Szeged. *21* (1—4), pp. 141—146 (1975)

# THE EFFECT OF SODIUM 2.4-DICHLOROPHENOXYACETIC ACID ON DOMESTICATED PLANTS

MÁRIA M. HORVÁTH and J. KERTÉSZ

*Genetics Group, Attila József University, Szeged*

(Received March 28, 1975)

## Abstract

In high concentration sodium 2.4-dichlorophenoxyacetic acid (2.4-D, Dikornit) inhibited the growth of experimental plants. Its effect also remains in the T generation. Increase of the 2.4-D concentration increased the amount of soluble protein and the activity of peroxidase (with the exception of maize). The hydroxylation processes too are influenced by 2.4-D, the amounts of the derivatives being increased at low concentration.

GRANT (1972) considers the herbicides to be local promoters of evolution. Even if only 1 p.c. of the domesticated plants undergoes significant change in a genetic balance on herbicide accumulation, it serves as the starting basis for another hereditary pathway. All this can be measured in the treated generations the structural and numerical changes in the cromosomes, and morphological, physiological and biochemical indices. The current increase of chemization in plant protection presents us with both theoretical and practical research tasks.

In the present paper a study is made of the effects of a herbicide, sodium 2.4-dichlorophenoxyacetic acid (2.4-D, Dikonirt) on some plant species. In accordance with their hormonal ground substance, at low concentrations the herbicides stimulate growth, and at high concentrations inhibit it (VEINBRANTS, 1972). WUU and GRANT (1966a, b) observed chromosomal aberrations in the meiosis of barley root-tips on the action of herbicides, and the pollen mother cells of the treated second generation also exhibited abnormal meiosis. When applied in various concentrations to wheat, sunflower and bean, 2.4-D enhances the activities of the enzymes participating in the oxidative metabolism (VORT, 1964).

## Materials and Methods

The experimental plants were MV 530 hybrid maize, broad bean, pea, pearl bean, cucumber, musk melon and water melon. The outdoor experiments were performed on 1 m plots. The 2.4-D dose was 1, 2, 4 or 10 g per plot for maize, and 0.05 or 0.26 g per plot for the other plants. The herbicide was employed as a preemergent simultaneously with the sowing, together with a sufficient number of control sowings. In the cytological and genetic examinations the plants were also grown in breeding vessels under semiconditioned circumstances for the observation of the development of the shaping of the genetic system variation. The controls were grown on tap-water and on 2.4-D solution with concentrations corresponding to those of the various outdoor experiments. A study was made of the modifying effect of 2.4-D, compared to the control, in the mitotic division, and of the changes occurring in the meiosis. The development of the protein content was measured by the method of LOWRY et al. (1951), and the changes in the pigments by the method of SMITH 1963). The peroxidase activity was determined by the procedure of SOLYMOSY—FARKAS (1964), and the indole hydroxylation was investigated by the method of HORVÁTH (1975).

## Results and Discussion

In the outdoor experiments it was observed that when 2.4-D was used as a preemergent herbicide the increase of its amount was paralleled by increasing inhibition or even complete cessation of the germination of the seeds. For maize, 10 g/m² was the highest dose applied, and this inhibited growth of the roots. The effect of 2.4-D was also apparent in the growth of the shoots and in the development of the number of leaves. The time of tassel-silking too was shifted, and the yield decreased. The one or two grains present were larger and heavier than the grain yield of the normally-developed control plant (KERTÉSZ, 1974).

The other experimental plants reacted with different sensitivities to the increase of the 2.4-D concentration. In these plants the highest concentration of the herbicide used was 0.25 g/m², and this completely inhibited the germination of musk melon and water melon seeds. Pearl bean, pea and broad bean plants grew to a lesser extent. The greater the amount of 2.4-D given in the preemergent stage, the more reduced was the length of the shoots and roots, and the fewer the leaves formed; further, these latter ones were more distorted in shape. Only a few seeds developed here too.

The gourds reacted with varying sensitivity to 2.4-D. Cucumber well endured even the highest 2.4-D concentration employed, and the growth and development of the shoots and roots were only slightly irregular. At a lower concentration no change could be observed compared to the control. On the contrary this same lower concentration led to much damage among musk melons and water melons, many plants dying before the reproductive stage. On this basis we see that there is a correlation between the water — demand and 2.4-D sensitivity, and this is why cucumber can well endure this herbicide. Our results are supported by the data in the following Table 1 measured on the meiosis of the cells of the root-tip meristem.

*Table 1.* Effect of 2,4-D on the meiosis of root-tip meristem cells of seedlings treated in the preemergent stage.

| Variant | No. of cells examined | No. of meiotic cells | Prophase | Metaphase | Anaphase | Telophase |
|---|---|---|---|---|---|---|
| *MV 530 maize* | | | | | | |
| Control | 2892 | 1068 | 906 | 51 | 57 | 50 |
| Low conc. | 2360 | 877 | 853 | 8 | 11 | 6 |
| High conc. | 2110 | 686 | 671 | 5 | 6 | 4 |
| *Pearl bean* | | | | | | |
| Control | 1160 | 521 | 278 | 98 | 78 | 67 |
| Low conc. | 547 | 41 | 11 | 13 | 9 | 8 |
| High conc. | 398 | 17 | 6 | 4 | 4 | 3 |
| *Broad bean* | | | | | | |
| Control | 448 | 196 | 171 | 9 | 6 | 10 |
| Low conc. | 1158 | 107 | 96 | 4 | 3 | 4 |
| High conc. | 1045 | 11 | 8 | 1 | 0 | 2 |

It can be seen from the Table that 2.4-D exerted a very considerable deceleratory influence on the course of the mitosis in all three plants, at the higher concentration almost eliminating the meiosis and thus the growth of the root.

The effect of 2.4-D could also be seen in the meiotic division of the $T_0$ plants treated. The arrangement of the chromosomes was irregular (ROJIK, 1973).

As regards the seeds of $T_0$ maize plants, it was found that the initial growth was more intensive than that of the control plants (KERTÉSZ, 1972).

The study of the $T_0$ and $T_1$ generations of broad bean led to a similar result as observed for maize. KOVÁCS (1972) found that 2.4-D is accumulated in the seeds. The extent of the accumulation rose with the increase of the concentration of the herbicide.

2.4-D inhibits the growth of seedlings; this is well illustrated in Table 2 by the higher amount of soluble protein in pearl-bean seedlings which underwent a slight degree of growth destruction.

*Table 2.* Change in the amount of total soluble protein in pearl bean seedlings on preemergent 2,4-D treatment (γ/g fresh weight).

| Age of seedling in days | Control | | | Treated with low conc. | | | Treated with high conc. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Coty-ledon | Shoot | Root | Coty-ledon | Shoot | Root | Coty-ledon |
| 3 | 4050 | 2205 | 4140 | 4120 | 2750 | 4400 | 4200 | 3850 | 5100 |
| 4 | 3375 | 1900 | 4320 | 3400 | 2600 | 4350 | 3800 | 3200 | 4300 |
| 5 | 3210 | 1885 | 4515 | 3170 | 2350 | 4010 | 3450 | 2840 | 4210 |
| 6 | 3105 | 1860 | 4650 | 2800 | 2250 | 3600 | 3260 | 2610 | 4130 |

Increase of the amount of 2.4-D led to a fall in the green pigment components of the experimental plants; this is connected with the hydrolysis of the proteins. There is barely any change in the amount of yellow pigment. These results were reported earlier (HORVÁTH, 1970). The herbicide increased the peroxidase activity in these plants, with the exception of maize seedlings. The results of the enzymatic measurements are shown in the following Table 3.

It can be seen that the peroxidase activity increased in parallel with the damage. Maize endured without damage the 2.4-D concentration employed here.

2.4-D causes damage in metabolism according to the above results, but it presumably also stimulates the functioning of an enzyme complex, as shown by the formation of hydroxy derivatives of indole. The derivatives are produced in larger amount at low 2.4-D concentration; at higher 2.4-D concentration the enzyme complex is directed to the elimination of the chemical, and because of this the amounts of the derivatives decrease. This was shown by the amounts of 5-hydroxyindole in the cases of broad bean, pearl bean and cucumber. The hydroxylation is intensive for both musk melon and water melon; various derivatives are formed, the amounts of these being lower at the low 2.4-D concentration. For pea a large amount of 5-hydroxyindole was formed in comparison to the control; higher concentration of 2.4-D decreased this amount.

*Table 3.* Change in peroxidase activity on preemergent 2,4-D treatment. (As percentage of control).

| Species and age in days | Treated with low conc. | | | Treated with high conc. | | |
|---|---|---|---|---|---|---|
| | Shoot | Root | Cotyledon | Shoot | Root | Cotyledon |
| Pearl bean | | | | | | |
| 4 | 110 | 118 | 254 | 135 | 214 | 281 |
| 6 | 121 | 192 | 62 | 228 | 432 | 218 |
| 8 | 125 | 405 | 85 | 334 | 667 | 177 |
| 10 | 128 | 471 | 105 | 334 | 667 | 145 |
| Cucumber | | | | | | |
| 6 | 100 | 91 | — | 100 | 121 | — |
| 16 | 96 | 108 | — | 122 | 191 | — |
| Musk melon | | | | | | |
| 7 | 144 | 267 | — | + | + | — |
| 14 | 181 | 318 | — | + | + | — |

+ Died

The variations in the natures and quantities of the hydroxyindoles are listed in Tables 4, 4/1 and 4/2.

*Table 4.* Changes in amounts of hydroxyindoles on the action of 2.4-D. (Derivatives given in γ/g fresh weight).

| Species | Treatment | Age in days | 5—OH—indole in | |
|---|---|---|---|---|
| | | | Shoot | Root |
| Pearl bean | Control | 4 | 2.15 | 1.25 |
| | Low conc. | | 1.10 | 1.10 |
| | High conc. | | 1.90 | 1.40 |
| Pearl bean | Control | 8 | 5.00 | 40.00 |
| | Low conc. | | 40.00 | 43.00 |
| | High conc. | | 3.00 | 4.00 |
| Pearl bean | Control | 12 | 3.50 | 3.00 |
| | Low conc. | | 17.00 | 20.00 |
| | High conc. | | 2.00 | 4.00 |
| Pea | Control | 7 | 4.65 | 33.00 |
| | Low conc. | | 20.00 | 30.00 |
| | High conc. | | 43.00 | 66.00 |
| Pea | Control | 14 | 4.60 | 56.00 |
| | Low conc. | | 28.00 | 56.00 |
| | High conc. | | 10.00 | 28.00 |

*Table 4/1.* Change in the amoung of 5-hydroxyindole on 2,4-D treatment (γ/g fresh weight).

| Broad bean | Shoot | | | Root | | |
|---|---|---|---|---|---|---|
| | Control | Low conc. | High conc. | Control | Low conc. | High conc. |
| $T_0$ generation | 16 | 4 | 4.5 | 5 | 4.5 | 28 |
| | Treatment of seeds* | | | Treatment of seeds* | | |
| $T_1$ generation | 4.5 | 12 | 11.5 | 12 | 12 | 17 |

* Part of the produce of the $T_0$ generation emerged unchanged in the $T_1$ generation; the indicated samples received a repeated 2,4-D treatment.

*Table 4/2.* Changes in the amounts of hydroxyindoles formed on the action of 2,4-D in cucumber, musk melon and water melon seedlings (γ/g fresh weight).

| Plant species and age in days | Indole deriv. | Shoot | | | Root | | |
|---|---|---|---|---|---|---|---|
| | | Control | Low conc. | High conc. | Control | Low conc. | High conc. |
| Cucumber | | | | | | | |
| 10 | 5-OH | 110.00 | 71.00 | 95.00 | 75.00 | 35.00 | 49.00 |
| 16 | 5-OH | 76.00 | 50.00 | 76.00 | 48.00 | 27.00 | 27.00 |
| 20 | 5-OH | 51.00 | 44.00 | 67.00 | 41.00 | 21.00 | 24.00 |
| Musk melon | | | | | | | |
| 6 | 4-OH | — | — | — | 135.00 | 120.00 | — |
| | 5-OH | — | — | — | 5.00 | 4.30 | — |
| 12 | 4-OH | 2.35 | 3.50 | — | 120.00 | 105.00 | — |
| | 5-OH | 2.60 | 2.00 | — | 3.00 | 3.00 | — |
| 20 | 4-OH | 2.30 | 3.00 | — | 4.90 | 27.00 | — |
| | 5-OH | 2.60 | 2.50 | — | 1.50 | 2.00 | — |
| Water melon | | | | | | | |
| 15 | 4-OH | 530.00 | 310.00 | — | 540.00 | 390.00 | — |
| | 5-OH | 52.00 | 41.00 | — | 57.00 | 40.00 | — |
| | 6-OH | 40.00 | 20.00 | — | 30.00 | 27.00 | — |
| 20 | 4-OH | 530.00 | 300.00 | — | 530.00 | 355.00 | — |
| | 5-OH | 65.00 | 20.00 | — | 60.00 | 21.00 | — |
| | 6-OH | 41.00 | 17.00 | — | 24.00 | 4.70 | — |

## References


GRANT, W. F. (1972): Pesticides — subtle promoters of evolution. — Symp. Biol. Hung. *12*, 43—50.

HORVÁTH, M.—LONTAI, I. (1970): Investigation of physiological changes in roots and shoots as a result of a herbicide treatment (II) Development of the pigment content of shoots. — Acta Biol. Szeged *16*, 95—98.

HORVÁTH, M.—GUBA, F.—HANUSZ, B.—KIRÁLY, L. (1975): Indol hidroxilálás különböző növényfajoknál (Indole hydroxylation in various plant species). —(in press). Acta Phytopathologica, Budapest.

KERTÉSZ, J.—MOLNÁR, A.—HORVÁTH, M. (1972): The effect of the sodium salt of 2,4-dichlorophenoxyacetic acid on the mitotic growth of yellow corn. — Acta Biol. Szeged *18*, 131—133.

KERTÉSZ, J. (1974): 2,4-diklórfenoxiecetsav Na sójának hatása kultúrnövények $T_0$, $T_1$ nemzedékének morfológiájára és anyagcseréjére (Effects of sodium 2,4-dichlorophenoxyacetic acid on the morphology and metabolism of To and $T_1$ generations of domesticated plants). — Doctoral dissertation, Szeged.

KOVÁCS, R. ZSUZSANNA (1972): Morfológiai és anyagcsereélettani vizsgálatok kultúrnövényeken 2,4-diklórfenoxiecetsav hatására (Morphological and metabolic-physiological examinations of the effects of 2,4-dichlorophenoxyacetic acid on domesticated plants). — Doctoral dissertation, Szeged.

LOWRY, O. H. — ROSELRENGHT, N. J. — FARR, A. L. (1951): Colorimetric determination of TCA precipitable protein content with a combined Folin-Biuret reaction. — J. Biol. Chem *139*, 265—275.

ROJIK, I. — HORVÁTH, M. — LONTAI, I. (1973): A herbicide effect in the meiosis of Vicia faba. — Acta Bot. Acad. Sci. Hung. *18*, 163—169.

SMITH, J. A. C. — BENITES, A. (1963): The protochlorophyll — chlorophyll transformation. The nature of protochlorophyll in leaves. — Cornegie Inst. Year Book *52*, 149—153.

VEINBRANTS, N. (1972): Effect of succinic acid-2,2-dimethyl hydrazide (Alar) or scoring on growth and flower initiation of young apple trees. — Aust. J. of Exptl. Agricult. and Animal Husbandry *12*, 88—95.

VORT, D. J. (1964): Effect of herbicides on plant composition and metabolism. — London—New York.

WUU, K. D. — GRANT, W. R. (1966a): Morphological and somatic chromosomal aberrations induced by pesticides in barley *Hordeum vulgare* L.). — Can. J. Genet. Cytol. *8*, 481—501.

WUU, K. D. — GRANT, W. R. (1966b): Induced abnormal meiotic behavior in a barley plant *Hordeum vulgare* L.) with the herbicide Lorox. — Fyton. Vicente. Coper. *23*, 63—67.

Adbress of the authors:
Dr. MÁRIA M. HORVÁTH
J. KERTÉSZ
Genetics Group,
A. J. University,
H—6701 Szeged, P. O. Box 428, Hungary

Acta Biologica Szeged. *21* (1—4), pp. 147—163 (1975)

# ANATOMICAL VARIATIONS AND PALAEOPATHOLOGICAL OBSERVATIONS IN PREHISTORIC SERIES

GY. FARKAS and ANTÓNIA MARCSIK

*Department of Anthropology, Attila József University, Szeged*

(Received October 15, 1974)

## Abstract

A study was made of anthropological finds mainly from the area of Southern Hungary, and dating from the Neolithic, inclusive of the Iron age. Seven anatomical variations, 12 other morphological variations, 2 congenital anomalies, exostoses, changes related with the circulatory system, pathological cases, tumours, traumas and dental diseases were diagnosed. Such phenomena were observed in a total of 95 individuals, in many cases several of the phenomena being found in a given individual. All this evidence indicates that even the populations living several thousand years ago included the most varied pathological cases, which appear to have increased in number as a consequence of the change-over to agriculture and the establishment of human settlements. Without the excavation of complete burial-grounds, it is not possible to give an exact and reliable answer to the question of the frequency of these phenomena in a population. With the observation and publication of the cases involved, it was intended to draw attention to phenomena of interest and importance from a medical-historical aspect, in finds originating from the Neolithic.

## Introduction

Very few palaeoanthropological finds exist, in which there are no lesions in at least one of the bones. The differentiation of the anatomical variations and congenital anomalies seems a relatively simple task, but the situation is much more difficult with the pathological cases. The study of these is unquestionably justified today in a palaeoanthropological evaluation, since they can provide valuable information regarding the biological structure of the population. Research of such a nature is hampered by two main problems primarily:

*a)* it is only in extremely rare cases that the research worker has the possibility to deal with the finds originating from excavation of a complete burial-ground, and consequently there is no way to observe the overall manifested change or variation;

*b)* establishment of an accurate diagnosis is particularly difficult in the case of the palaeopathological changes.

We have tried to solve these two problems by incorporating into the investigation a large set of finds from an archaeological period which have so far not been reported, while in addition we have taken into account publications which contain detailed descriptions and good illustrations with regard to the changes in question.

## Examination material

A study was made of those skeletons (mainly crania) from the palaeoanthropological collection of the Department of Anthropology of Attila József University, which originate from authenticated sites dating from the Neolithic, inclusive of the Iron Age. The finds in which some change was observed are listed briefly below. The scope of this paper does not permit us to give an exact description of every individual skeleton; accordingly, the changes have been numbered (from 1 to 45), and in the treatment of the individual finds and of the changes the relavant symptoms are indicated by the appropriate number in brackets. At the same time, the most typical cases are presented in photographs.

Neolithic age

A. Kőrös group

No. 1936. Endrőd. Excavated by Ferenc Móra in 1930. Mature male. (22, 42 Taxon: n—m.

No. 131. Grave 25/1. Hódmezővásárhely-Kotacpart-Vata farm (BANNER, 1935). Adult male. (10, 40) Taxon: pn-x.

B. Tisza culture

No. 161. Grave 2. Hódmezővásárhely—Kökénydomb—Szabó (Kapocsi) farm (BANNER, 1951). Adult female. (26, 42). Taxon: am-pn.

No. 159. Hódmezővásárhely—Kökénydomb—Vörös farm (BANNER, 1940). Juvenile-adult female. (2, 2c, 3).

No. 4392. Grave 2. Nádudvar—Farkaslóré—Vörös Csillag Cooperative Farm (GAZDAPUSZTAI, 1963). Child of Infantia II age. (2, 29).

No. 4054. Grave 4. Hódmezővásárhely—Gorzsa—Czukor farm (GAZDAPUSZTAI, 1963a). Adult female. (42). Taxon: am-crA.

No. 4056. Grave 7. Hódmezővásárhely—Gorzsa—Czukor farm. Juvenile female. (29).

No. 7963. Grave 1. Vésztő—Mágori Hill (HEGEDŰS, 1973). Adult female. (3, 38). Taxon: n.

No. 7966. Grave 4. Vésztő—Magori Hill. Adult female. (43). Taxon: am-x.

No. 7968. Grave 6. Vésztő—Mágori Hill. Mature female. (1a, 38, 42). Taxon: n-x.

No. 7969. Grave 7. Vésztő—Mágori Hill. Mature male. (5a, 42). Taxon: n-am.

C. Neolithic finds not classified into ages

No. 3955. Mogyorós. Excavated by Ferenc Móra in 1930. Adult female. (3a, 43). Taxon: n-crB.

No. 3510. Grave A. Ószentiván VIII. (B. KUTZIÁN, 1961). Senile male. (21, 32 37). Taxon: pn-x.

Copper Age

A. Tiszapolgár culture

No. 1948. Grave 12. Deszk-A (FOLTINY, 1941). Mature male. (2). Taxon: am-pn.

No. 102. Grave 7/15. Hódmezővásárhely—Kotacpart—Vata farm. 1932—33 (B. KUTZIÁN, 1972). Senile male. (22). Taxon: pn.

No. 104. Grave 7/17. Hódmezővásárhely—Kotacpart—Vata farm, 1932—33. Senile female. (11). Taxon: crA-x.

No. 140. Sporadic. Hódmezővásárhely—Kotacpart—Vata farm, 1933—34 (BANNER, 1933—34). Mature-senile male. (2, 3a). Taxon: a-crB.

No. 1934. Grave 4. Lebő-A Farkas farm (B. KUTZIÁN, 1972). Mature male. (3a, 22, 31, 35). Taxon: pn-x.

No. 1433. Grave 1. Ószentiván (TÓTH, 1942). Juvenile female. (2). Taxon: am-x.

No. 3508. Grave 2. Ószentiván VIII (B. KUTZIÁN, 1961). Senile male. (2, 2c, 3, 8, 22a). Taxon: am-x.

B. Bodrogkeresztúr culture

No. 437. Grave 2. Magyarteés (PATAY, 1943). Adult male. (39).

No. 438. Grave 6. Magyarteés. Senile male. (23, 42). Taxon: crA-pn.

No. 1025. Designation 7. Szentes—Kistőke—Szegi farm (ZALOTAY, 1933—34; PATAY, 1943). Adult female. (2, 5a). Taxon: a-crB.

C. Pécel culture

No. 2664. Grave 8. Baja—Dózsa György St. 233 (KŐHEGYI, 1961). Senile male. (3a). Taxon: pn-crA.

Bronze Age

A. Early Bronze Age

No. 123. Röszke. Senile male. (23, 32, 35).

No. 3619. Grave 138. Szolnok—Rákóczifalva—Kastélydomb (CSALOG, 1963). Senile male. (17, 43). Taxon: crB-crA.

No. 170. Grave 10. Szőreg-C (FOLTINY, 1941a). Adult male. (2, 29, 31). Taxon: am-pn.

No. 174. Grave 20. Szőreg-C. Senile female. (31, 32). Taxon: n-m.

No. 176. Grave 24. Szőreg-C. Child of Infantia II age. (29).

No. 180. Grave. 29. Szőreg-C. Mature male. (11). Taxon: am-m.

No. 183. Grave 36. Szőreg-C. Senile male. (1, 23). Taxon: pn-x.

No. 197. Grave 62. Szőreg-C. Mature male. (2). Taxon: n-crB.

No. 207. Grave 74. Szőreg-C. Adult male. (2, 3a, 5, 15, 43). Taxon: am-pn.

No. 229. Grave 103. Szőreg-C. Child of Infantia II age. (25).

No. 241. Grave 120. Szőreg-C. Adult male. (18). Taxon: pn-crA.

No. 251. Grave 135. Szőreg-C. Mature female. (2, 27). Taxon: am-crA.

No. 1737. Szőreg—Pálfy brickworks. Adult male. (3a, 9, 19, 22, 24, 36). Taxon: pn.

B. Middle Bronze Age.

No. 4039. Grave 63. Deszk-A (FOLTINY, 1941). Adult male. (2). Taxon: am-m.

No. 1615. Grave 81a. Deszk-A. Mature female. (2, 5b, 22, 44). Taxon: d-p.

No. 2410. Grave 8. Deszk-F (FOLTINY, 1942). Adult male. (3a, 14,42). Taxon: am-crB.

No. 2414. Grave 14. Deszk-F. Adult male. (16).

No. 2415. Grave 15. Deszk-F. Adult female. (2, 3). Taxon: crB-am.

No. 327. Grave 26. Deszk-F. Adult male. (23).

No. 328. Grave 31. Deszk-F. Senile male. (23, 43). Taxon: n-x.

No. 329. Grave 32. Deszk-F. Mature male. (23, 43). Taxon: am-x.

No. 331. Grave 41. Deszk-F. Senile female. (23, 32, 43).

No. 332. Grave 45. Deszk-F. Adult female. (43). Taxon: a-crB.
No. 333. Grave 46. Deszk-F. Adult male. (2, 3, 5, 7, 22, 29). Taxon: n-am.
No. 337. Grave 60. Deszk-F. Mature male. (23, 35, 43). Taxon: pn-m.
No. 338. Grave 63. Deszk-F. Adult male (?). (2, 22). Taxon: a-x.
No. 339. Grave 64. Deszk-F. Mature female. (32).
No. 340. Grave 67. Deszk-F. Mature male. (23, 24, 31). Taxon: d.
No. 341. Grave 68. Deszk-F. Adult male. (23). Taxon: am-x.
No. 169. Grave 9. Szőreg-C (FOLTINY, 1941a). Senile female. (28). Taxon: pn-x.
No. 172. Grave 13. Szőreg-C. Adult female. (2, 5a, 22). Taxon: am-crB.
No. 177. Grave 26. Szőreg-C. Adult male. (2, 3a, 5a, 11). Taxon: n-crA.
No. 182. Grave 35. Szőreg-C. Senile male. (43). Taxon: crA-a.
No. 198. Grave 64. Szőreg-C. Adult female. (29). Taxon: am-m.
No. 201. Grave 67. Szőreg-C. Mature male. (39). Taxon: am-crA.
No. 214. Grave 84. Szőreg-C. Mature male. (3a). Taxon: pn-am.
No. 216. Grave 86. Szőreg-C. Mature female. (43). Taxon: am-n.
No. 235. Grave 109. Szőreg-C. Adult female. (2, 3, 10, 42, 43). Taxon: am-n.
No. 236. Grave 110. Szőreg-C. Adult male. (1, 43). Taxon: crA-x.
No. 252. Grave 136. Szőreg-C. Adult male. (2, 20, 42, 43). Taxon: pn-am.
No. 254. Grave 138. Szőreg-C. Mature female. (29, 40, 42, 43). Taxon: crB-am
No. 258. Grave 146. Szőreg-C. Adult male. (2, 6). Taxon: am.
No. 270. Grave 166. Szőreg-C. Child of Infantia II age. (2, 2a, 2b, 2c, 4, 13, 29)
No. 271. Grave 167. Szőreg-C. Child of Infantia I age. (29, 30).

C. Late Bronze Age

No. 325. Grave 22. Deszk-F (FOLTINY, 1942). Senile male. (3a, 22, 23, 43). Taxon: n-crA.

No. 3558. Grave 57. Szolnok—Rákóczifalva—Kastélydomb (CSALOG, 1963). Adult male. (2, 31).

No. 3631. Grave 155. Szolnok—Rákóczifalva—Kastélydomb. Adult male. (43). Taxon: m-n.

No. 3636. Grave 163. Szolnok—Rákóczifalva—Kastélydomb. Adult female. (2, 42).

No. 209. Grave 76. Szőreg-C (FOLTINY, 1941a). Mature female. (9, 42). Taxon: am-m.

No. 219. Grave 89a. Szőreg-C. Adult female. (45). Taxon: am-crA.
No. 220. Grave 89b. Szőreg-C. Child of Infantia II age. (2, 29).
No. 237. Grave 112. Szőreg-C. Mature male. (2, 5a, 43). Taxon: p-crB.
No. 257. Grave 144. Szőreg-C. Senile female. (43). Taxon: a-crB.
No. 266. Grave 160. Szőreg-C. Senile female. (32).

D. Bronze Age finds not classified into ages

No. 5284. Grave 1. Hódmezővásárhely—State Farm. Adult male. (2, 23, 41).

No. 5285. Hódmezővásárhely—State Farm. Mature male. (3, 23, 33, 38). Taxon: pn-crA.

No. 3561. Grave 63. Szolnok—Rákóczifalva—Kastélydomb (CSALOG, 1963). Mature male. (2, 2a, 22, 42, 43). Taxon: n-am.

No. 3599. Grave 110. Szolnok—Rákóczifalva—Kastélydomb. Mature female. (2).

No. 3507. Szolnok—Waterworks. Mature male. (23, 30).

No. 190. Grave 54. Szőreg-C (FOLTINY, 1941a). Mature male. (2, 12, 38, 43). Taxon: crB-n.

No. 224. Grave 97. Szőreg-C. Adult female. (3, 5a 42, 43). Taxon: a-crA.

No. 274. Szőreg-C. Adult male. (3a, 24, 29, 31). Taxon: m-crA.

Iron Age

No. 3175. Grave 1. Csanytelek. Adult female. (2, 5b, 10).

No. 3176. Grave 2. Csanytelek. Mature male. (2). Taxon: crB-x.

No. 3177. Grave 3. Csanytelek. Child of Infantia II age. (35).

No. 3178. Grave 4. Csanytelek. Adult female. (2). Taxon: crB-a.

No. 4049. Grave 3. Kishomok—Lenin Cooperative Farm (GAZDAPUSZTAI, 1964). Adult female. (3, 31). Taxon: am-n.

No. 1429. Grave 1. Lebő (PÁRDUCZ, 1942). Mature male. (22, 23, 34). Taxon: n-am.

No. 1430. Grave 2. Lebő. Senile male. (5a, 22b, 42, 43). Taxon: crA.

No. 368. Grave 1. Szentes—Vekerhát (PÁRDUCZ, 1940). Mature female. (23, 42, 43). Taxon: crB-x.

No. 434. Grave 1. Szentes—Vekerzug—Bleierföld. Excavated by Gábor Csallány. Adult female. (2, 38). Taxon: a-crB.

The taxon establishment was carried out by the method of LIPTÁK (1962, 1965, 1971), according to the average values elaborated (FARKAS, 1972) on the basis of Lipták's reported finds. In accordance with this, the letters used for taxon designations have the same sense as given by LIPTÁK.

Tables 1—3 provide information on the distributions of the above finds according to sex, age of death, taxon and archaeological period.

*Table 1.* Distributions of the examination series according to sex and archaeological age.

| Archaeological age | | Male | Female | Children | Total |
|---|---|---|---|---|---|
| Stone Age | Körös group | 2 | — | — | 2 |
| | Tisza culture | 1 | 7 | 1 | 9 |
| | Unclassified | 1 | 1 | — | 2 |
| | Total | 4 | 8 | 1 | 13 |
| Copper Age | Tiszapolgár | 5 | 2 | — | 7 |
| | Bodrogkeresztur | 2 | 1 | — | 3 |
| | Pécel | 1 | — | — | 1 |
| | Total | 8 | 3 | — | 11 |
| Bronze Age | Early | 9 | 2 | 2 | 13 |
| | Middle | 18 | 11 | 2 | 31 |
| | Late | 4 | 4 | 1 | 9 |
| | Unclassified | 6 | 3 | — | 9 |
| | Total | 39 | 20 | 5 | 62 |
| Iron Age | | 3 | 5 | 1 | 9 |
| Total | | 52 | 36 | 7 | 95 |

It is quite clear from Table 1 that on the occasion of the recovery of the various finds the skeletons of children were recovered only in low numbers. Correspondingly, very few child-skeletons occur among the examination series.

Table 2 reflects the well-known relation that there is a significant difference between the ages of death for males and females. In this series too it is observed that the number of males of mature and senile age (32) is well in excess of the corresponding number of females of the same age (15).

*Table 2.* Distributions of the examination series according to sex and age.

| Age | Male | Female | Children | Total | |
|---|---|---|---|---|---|
| | | | | n | % |
| Infantia I | — | — | 1 | 1 | 1.0 |
| Infantia II | — | — | 6 | 6 | 6.3 |
| Juvenile | — | 3 | — | 3 | 3.2 |
| Adult | 21 | 18 | — | 39 | 41.1 |
| Mature | 19 | 9 | — | 28 | 29.5 |
| Senile | 12 | 6 | — | 18 | 18.9 |
| Total | 52<br>54.7% | 36<br>37.9% | 7<br>7.4% | 95 | |

Table 3 gives the distribution according to taxon. It well illustrates the proportions of human species to be observed in prehistoric material. Thus, in addition to the 26 Mediterraneans, 26 nordoid, 12 cromagnoid and 10 brachycephalic taxons

*Table 3.* Distribution of the examined adult finds according to taxon and sex.

| Taxon | Male | Female | Totale | |
|---|---|---|---|---|
| | | | n | % |
| Nordoids: | | | | |
| Protonordic (pn) | 12 | 1 | 13 | 14.7 |
| Nordic (n) | 9 | 4 | 13 | 14.7 |
| Cromagnoids: | | | | |
| Cromagnoid-A (crA) | 4 | 1 | 5 | 5.6 |
| Cromagnoid-B (crB) | 3 | 4 | 7 | 7.9 |
| Mediterraneans: | | | | |
| Gracile mediterranean (m) | 2 | — | 2 | 2.2 |
| Atlantomediterranean (am) | 11 | 12 | 23 | 26.1 |
| Brachycephalics: | | | | |
| Alpine (a) | 2 | 5 | 7 | 7.9 |
| Dinarian (d) | 1 | 1 | 2 | 2.2 |
| Pamirian (p) | 1 | — | 1 | 1.1 |
| Undetermined | 7 | 8 | 15 | 17.0 |
| Total | 52 | 36 | 88 | |

occured, which is primarily correlated with the southern origins of the prehistoric population. A difference in taxonomic composition between the two sexes can be seen essentially only in case of the nordoid group, for there are almost four times as many of these among the males as among the females.

## Anatomical variations, congenital anomalies

The anatomical variations are the results of changes occurring during the ossification process, and the heredity-course of these is not known exactly. BROTHWELL (1959) proposes the examination of 10 (non-metric) features to demonstrate the biological distance between populations.

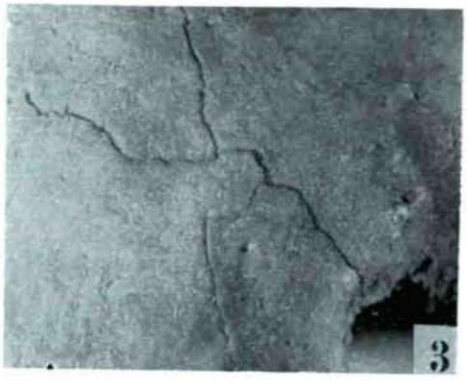


Fig. 1. Szőreg-C, early Bronze Age, No. 183, Grave 36. — Large uniform os apicis.
Fig. 2. Szőreg-C, middle Bronze Age, No. 270, Grave 166. — Wormian bone in the sutura sagittalis.

The os apicis is a uniform or divided bone situated in the sutura lambdoidea, in the interparietal angle of the os occipitale (MARTIN—SALLER, 1957—1966). It occured as a uniform bone (1) in two finds from Szőreg (Fig. 1), but the variant divided into three parts (os apicis tripartium) (1a) was also observed in one cranium.

The wormian bones are larger or smaller bone-islands situated in the sutures HESS, 1946; MARTIN—SALLER, 1957—1966; KENNETH, 1965). Of the anatomical variations examined, this was found with the highest frequency. The distribution according to sex of the wormian bone occurring in the sutura lambdoidea (2): 18 males, 13 females and 3 children. It was found in the sutura coronalis (2a) in two cases. In one find it was observed in the pars obelica of the sutura sagittalis (2b) (Fig. 2), and was also to be seen in the sutura parietomastoidea (2c). Wormian bones in similar sites were found in a further two crania.

The sutura metopica (3) is a persisting foetal frontal suture, which normally occludes during the first two years after birth (ESSEN—HÖLLER, 1928). This was found in 9 crania (3 males, 6 females) among our material. The supranasal sutural residue (3a), which is a residue of the foetal frontal suture (MARTIN—SALLER, 1957—1966), was observed in 11 crania (10 males, 1 female).



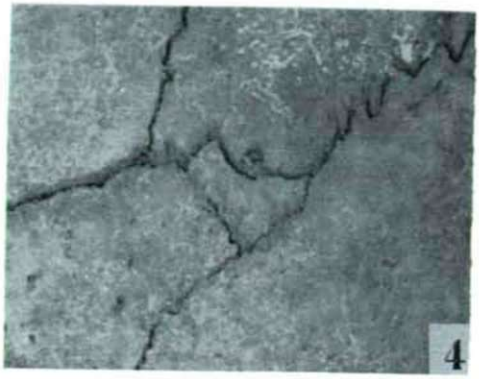


Fig. 3. Szőreg-C, middle Bronze Age, No. 270, Grave 166. — Right-side processus frontalis ossis temporalis.
Fig. 4. Szőreg-C, late Bronze Age, No. 237, Grave 112. — Os epiptericum.

Of the variations of the pterion region (MARTIN—SALLER, 1957—1966), the processus frontalis ossis temporalis (4) occurred on the right side in the cranium of one child (Figs. 3 and 7). A fairly frequent variation of this region is the appearance of an independent bone, the os epiptericum, which is generally regarded as the wall bone of the temporal bone (MARTIN—SALLER, 1957—1966). This was observed in 6 males, in 2 on both sides (5) and in 4 (Fig. 4) only on the left side (5a), and also in 5 females, in 3 on the left side and in 2 on the right side (5b).

The os bregmaticum (6) is a fontanelle which occurs partly symmetrically, and partly asymmetrically in the initial section of the sutura sagittalis, but it is also found extending into the frontal bone. Its appearance is relatively rare (MARTIN—SALLER, 1957—1966). It is restricted to one case in our material (Fig. 5).

The processus paramastoideus or paracondyloideus (7) is an extremely rare variation (MARTIN—SALLER, 1957—1966), and similarly occurred in only one case in our material.

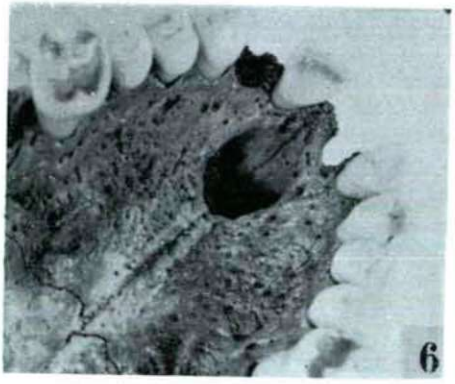


Fig. 5. Szőreg-C, middle Bronze Age, No. 258, Grave 146. — Os bregmaticum
Fig. 6. Hódmezővásárhely—Kotacpart—Vata farm, early Neolithic (Kőrös group), No. 131. Grave 25/1. — Large foramen incisivum

Variations of other morphological characteristics were also observed:

the left-side canalis nervi hypoglossi (8) was larger than the average in one case;

supernumerary right-side foramen infraorbitale (9) was found in two cases;

a large foramen incisivum (10) could be seen in three cases (Fig. 6). In such a case the possibility of a cyst may come to mind (BALOGH, 1958);

the right-side foramen jugulare was larger (11) than the left in three cases. The enlargement of the right foramen may well be indicative of the left-handedness of the individual, for the pathological change can be assumed due to the over-narrowing of the left foramen (CHARVART—PACOVSKY—DUBOVSKY, 1964);

foramen jugulare bipartitum (12) can be seen on both sides in the cranium of one male;

the fissura sphenopetrosa is strikingly wide (13) in the case of one child. Since, apart from the crossing of the nervus petrosus superficialis major and minor, this fissure also acts as an emissary (KISS, 1953), in this child the veins in this region may have been larger or supernumerary;

the partition of the left capitulum mandibulae (14) occurred in one case;

similarly, bipartition of the right ala magna (15) was observed in one case (Fig. 7);

the left angle of the mandibula was inclined inwards markedly (16) in one male;

the crista occipitalis externa appeared in the form of a strong ridge (17) in one cranium;

the spina mentalis was unusually strongly developed (18) in one case. Muscle-adhering bone fissures with abnormal development are in a causel relation generally with a musculature hypertrophized because of over-stressing. One form of this is strong development of the spina mentalis (REGÖLY—MÉREI, 1962);

the right foramen mentale is larger (19) than the left in one find.

Of the congenital anomalies, mention may be made of the condylus tertius (20), which was found in one case (Fig. 8).

Assimilation of the os coccygis (21) was observed in the sacrum of one male.

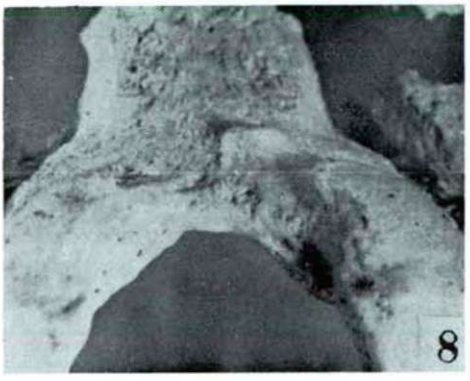




Fig. 7. Szőreg-C, early Bronze Age, No. 207, Grave 74. — Right-side os epiptericum and divided ala magna.

Fig. 8. Szőreg-C, middle Bronze Age, No. 252, Grave 136. — Condylus tertius.

## Exostoses

The esoxtoses can be observed in the region of the palatum durum (22), mainly near the molars, in the form of larger or smaller ridges, but they may also occur on the left ramus mandibulae (22a), as was observed on the lower mandible of one male. A similar case may also appear on the ala magna (22b) (one case).

Of the total of 13 exostoses observed (including the two mentioned), with the exception of two females all occurred in male finds. With one exception, advanced dental abrasion too was found in these. It is seen that this may be connected with the oral cavity and masticatory muscles, and because of this the fairly frequent occurrence may be explained by over-loading (REGÖLY—MÉREI, 1962). This assumption is contradicted, however, by cases of crania where the masticatory surface on the teeth is very worn, but at the same time exostosis is not found on the palatum durum.

## Changes related with the circulatory system

The abnormal number, position and size of formations (foveoles granulares, Pacchioni granules, sulci arteriosi) on the endocranial surface of the crania were also examined (23).

The Pacchioni granules are the formations of the arachnoidea, which are papilla-like prominences on both sides of the fissura interhemisphaerica. They penetrate into the venous sinuses of the dura mater. In old age they increase in number, and thus the number of indentations corresponding to the Pacchioni granules also rises. The granules have the function of acting as baroreceptors to measure the pressure of the cerebrospinal fluid. Those situated farther from the mesial line also penetrate into venous lacunae which are in connection with the sinuses. Corresponding to these large lacunae, smaller indentations (foveoles granulares) are found on the parietal bones (KISS, 1953; RATKÓCZY, 1959). These changes have also been demonstrated in palaeoanthropological material (NEMESKÉRI—HARSÁNYI—GERENCSÉR, 1973). As regards the sulci arteriosi in another site (REGÖLY—MÉREI, 1962) it was found that the broadened and deepened vascular sulci may appear as a consequences of an intracranial pressure increase.

The frequency of combined occurrence of these formations too was examined, but only the individuals were regarded as pathological who belonged to the adult age group (since the number of Pacchioni granules rises with age). A larger than average number of Pacchioni granules with a deep vascular sulcus appeared in the case of two adult males; supernumerary foveoles granulares and a deep vascular sulcus were observed on the endocranial surface of one male cranium. Similar changes were found in a further 13 individuals (11 males and 2 females), all of them belonging in the mature or senile age groups.

Special attention should be paid to a fine network of thin vascular sulci (24) on the margo supraorbitalis and the arcus superciliaris (3 individuals). Changes of this nature were observed on prehistoric men finds by TAPPEN (1973).

## Other pathological changes

Synostosis praecox (25) is the premature synostosis of the cranial bones, primarily along the sutura sagittalis and coronalis (BARTUCZ, 1966). In our material this could be observed on the region of the sutura sagittalis in one child (of Inf. II age).

Less serious hydrocephalus (26) is indicated by the large cranium of an adult female. Such changes have previously been described in a palaeoanthropological material (BROTHWELL, 1965).

The osteomas (27) are slowly-growing, benign bone tumours. They may also appear on the calvaria, customarily in the form of small, round protuberance (HARANGHY, 1966). A protuberance about half a centimetre in diameter was observed on the frontal bone of one individual. Osteoma agreeing with our case is found in the work of VYHNÁNEK (1971).

A particularly great problem was caused by a round foramen (28), approximately half a centimetre in diameter, situated in the upper lateral wall of the orbit of one cranium and in the spongiosa. The opening is surrounded by the sulci of a rich vascular network. No other changes can be observed in the cranium, and the indentation penetrating into the spongiosa is not in connection with the sinus frontalis. Since a change of a quite local nature is involved and the literature shows no photographs relating to this phenomenon, we thought of the following aetiological factors:

*a)* some change of the glandula lacrimale, which induced a secondary bone destruction;

*b)* angioma, which according to REGÖLY—MÉREI (1962) can also arise in the medulla, mainly in the form of haemangioma capillare. The various forms of haemangioma can also occur in the adipose tissue (orbit) (HARANGHY, 1966);

*c)* orbital infection.

The foramination of the upper medial wall of the orbital tegmen was termed by WELCKER (1888) cribra orbitalia (29). The osteoporotic area occurring in the parietal region of the calvaria (when the tabula externa disappears and the enlarged spongiosa becomes visible on the surface) is usually referred to as hyperostosis spongiosa cranii (HAMPERL—WEISS, 1955) (30). Our evaluations of these phenomena were based on the work of other authors (NATHAN—HAAS, 1966). Because of the low number of cases a difference in sex could not be established, but at any event it is striking that there are a significant number of child-crania exhibiting the change. Three basic types were observed (NATHAN—HAAS, 1966): porotic, cribrotic and trabecular. Accordingly, the following cases occurred in our examination material: porotic change in 1 male, 2 females and 1 child; cribrotic change in 1 male, 2 females (Fig. 9) and 3 children. The cribra orbitalia of one child forms a transition between the cribrotic and trabecular forms. In this case a 3×2 cm elliptic hyperostosis spongiosa cranii too was seen on the os parietale. Besides cranium no. 3507 from the area of the Szolnok Waterworks, parietale was also found in another individual. Here a circular osteoporotic area, 1.5 cm in diameter, can be seen on the region enclosed by the sutura sagittalis and coronalis, with hyperostosis of the spongiosa. The majority of research workers today agree that the factors responsible for the development of this process are:

*a)* malnutrition (HENSCHEN, 1961);

*b)* iron deficiency anaemia accompanying malnutrition (NATHAN—HAAS, 1966);

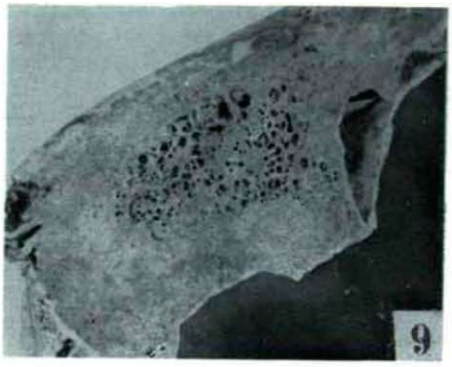
*c)* iron deficiency anaemia (HENGEN, 1972; CULE—EVANS, 1968);

*d)* haemolytic anaemia (ANGEL, 1966).

The differentiation of these in historical material is extremely problematical, whereas the recognition of the changes is relatively easy (BRABANT—NEMESKÉRI, 1963).

Changes of a local osteoporotic nature (31), which appear in the form of a fine foramination, were observed on the various parts of the cranium in several cases:
on the area of the arcus superciliaris and the glabella (4 cases);
on the arcus superciliaris and the arcus zygomaticus (1 case);
on the area of the porus acusticus externus and the condylus occipitalis (1 case);
on the ala magna (1 case).

A special classification was made of the crania which can be regarded as examples of senile osteoporosis (32) (PRATOP, 1969). Its typical appearance form is atrophy of the calvaria. It was observed in a total of 5 female cases, all of them from the mature or senile age groups. Mention must also be made here of the skeletal bones of a senile male, which were found to be unusually light, while the marks of absorption too were to be seen on the long tubular bones and on the pelvis.



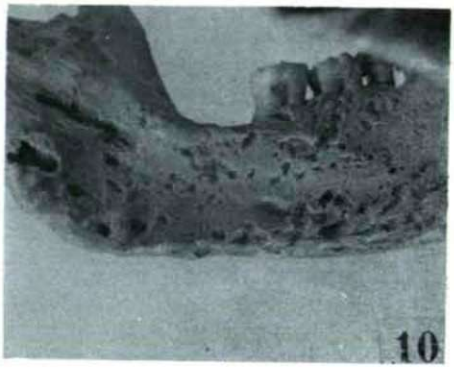




Fig. 9. Hódmezővásárhely—Gorzsa—Czukor farm, Neolithic (Tisza culture), No. 4056, Grave 7. — Cribra orbitalia (cribroticus).
Fig. 10. Lebő, Iron Age, No. 1429, Grave 1. — Myeloma multiplex on the mandible.

## Tumours

Meningeoma (33) is a tumour of the cover cells of the arachnoidea, which causes an impression in the cerebral substance and indicates hyperostosis in the adjacent bone tissue (ENDES, 1972). The explanation of this is that, because of the pressure increase due to the tumour, hyperaemia develops in the diploe with the mediation of the ample vascular connections between the dura and the diploe (REGÖLY—MÉREI, 1962). The new bone formed is compact, structureless, but possibly with a radial structure (RATKÓCZY, 1959). Meningeoma has also been mentioned in a prehistoric context (JANSSENS, 1970). On the basis of the above description, meningeoma was assumed for the changes of one male cranium in our material. In this the parietal bone attained a considerable thickness in the corresponding area of the tubera frontalia and in the occipital region; this is shown by the local thickening of the spongiosa. In the region corresponding to the course of the lambdoid suture this thickening resulted in bathrocephalus. The cranium is extremely light. The condylus occipitalis is not parallel with the basal plane and the fossa condyloidea is extremely deep. The X-ray picture shows only the parietal bone

thickening. (We express our thanks to Dr. LÁSZLÓ PÁLDY for the preparation of the picture.)

Myeloma multiplex (34) is a tumour starting out from the reticulum of the medulla, which breaks through the compact substance of the bone to reach the surface (HARANGHY, 1966). It appears in the form of round, sharp-edged bone defects (RATKÓCZY, 1959). Such bone lesions have been reported by Hungarian authors earlier (NEMESKÉRI—HARSÁNYI, 1959). Taking into account another description too (WILLIAMS—RITSCHIE—TERRINGTON, 1941), we explain the cause of such changes in one male cranium on the basis of the photographs (Fig. 10) as myeloma multiplex.

Indentations (35) can be seen on the endocranial surface of four crania (in one case protruding onto the ectocranial surface too), mainly along the sutura sagittalis, in the area of the parietale or the occipitale. In these areas the parietal bone is thinned. In our view some intracranial tumour may be involved, although leptomeningitis too causes indentations in the cranium (WADSWORTH SCHWARTZ, 1941).

Degenerative changes of the articular surfaces of the vertebrae are generally described as spondylarthrosis (36) (VYHNÁNEK, 1971, ACSÁDI—HARSÁNYI—NEMESKÉRI, 1962). On one cranium small exostoses were seen on the anterior edge of the foramen magnum, and on the basis of the flattened state of the condylus occipitalis the phenomenon (Fig. 11) was explained as spondylarthrosis. (Unfortunately, the atlas is missing.)

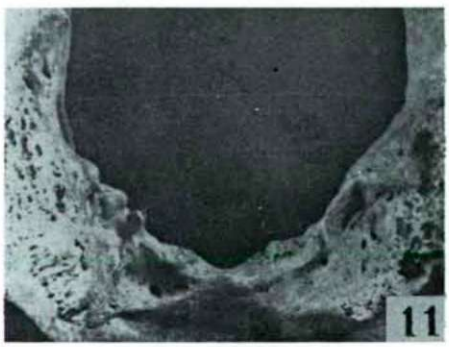




Fig. 11. Szőreg—Pálfy brickworks, early Bronze Age, No. 1737. — Traces of Spondylarthrosis on the condylus occipitalis.

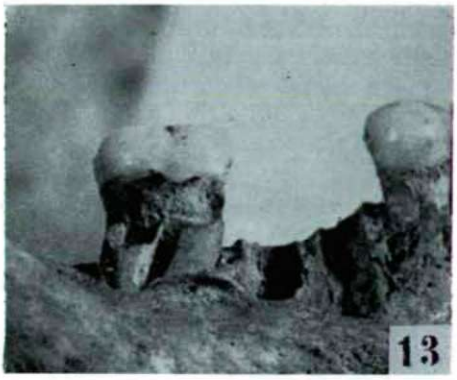
Fig. 12. Vésztő—Mágori hill, Neolithic (Tisza culture), No. 7963, Grave 1. — Injury resulting from a stab (?) on the mandible.

A similar change was seen in the vertabrae of another individual. In this case the degenerative process affected the bodies of the vertebrae. Strong osteophyte formation can be observed on the bodies of the thoracales and lumbales, but less so on the vertebrae cervicales; in the cases of thoracic vertebrae 9 and 10 it can be seen that the two vertebrae are fixed with boney bridges (VYHNÁNEK, 1972). In the literature this osteophyte formation is generally described as spondylosis deformans (37) (NEMESKÉRI—HARSÁNYI, 1959; TULSI, 1972).

## Traumas(38)

In several cases the crania exhibited larger or smaller changes indicative of external effects: the mark of a sharp weapon on the os frontale; the deep furrow of the penetration of an arrowhead (?) on a mandible (Fig. 12); in one case the mark of a stronger blow (?) on the area of the os frontale, and in two cases wounds on the top of the head, resulting from blows (?) on the area of the os frontale or parietale.

We do not deal separately with the question of trepanations (39), as these cases already figure in earlier publications (BARTUCZ, 1966).



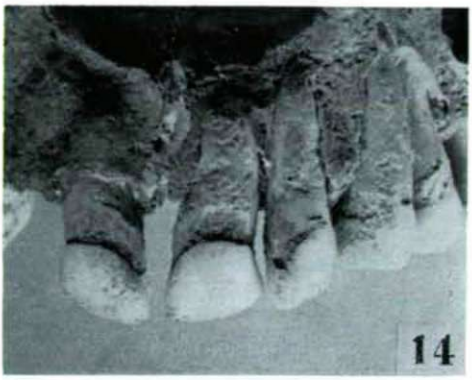




Fig. 13. Szőreg-C, middle Bronze Age, No. 254, Grave 138. — Cercival caries at $M_2$.

Fig. 14. Szőreg-C, middle Bronze Age, No. 254, Grave 138. — Cement-enamel caries on the incisors.

## Teeth

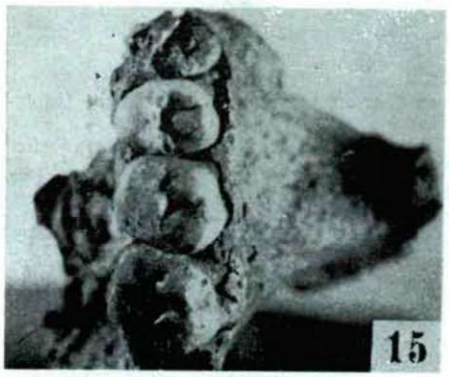
The degree of cariosity on teeth increases in historical times up to the present day. This was already studied in the series under examination here (TÓTH, 1970; TÓTH—SONKODI, 1972). In this respect we refer to these publications. It must be mentioned, however, that in our judgement a study of the frequency of caries (40) in teeth can lead to a realistic result only if the existing teeth of a given type are examined and the caries frequency is expressed as a percentage of the cases examined. Research into the problem is highly justified, for even in finds which are several thousand years old an extremely high degree of caries is found on various sites of the dental crown and root (Figs. 13 and 14).

In connection with the teeth we consider it necessary to turn the rare cases too which occur in prehistoric series. We think primarily of the only supernumerary molar (hyperodontia) (41), presented in Fig. 15.

Diseases of the teeth or periodontium can give rise to abscess or granuloma around the root (REGÖLY—MÉREI, 1962). With its slow growth the granuloma destroys the bone around the root apex, and even the root apex itself(BALOGH, 1958). The cysts are generally larger than the abscess cavities, and round or elliptic in shape (BALOGH, 1958). Their differentiation in historical material means a certain difficulty, and thus in our cases (Fig. 16) we give the combined number of granulomas and cysts (42) present. Fistula too was found in 2 of the 16 individuals; this can occur

as a result of periapical abscess, osteomyelitis, or maxillary sinus suppuration of dental origin (REGÖLY—MÉREI, 1962).

Osteomyelitis of dental origin (43) is either localized or extensive. The osseous surface of the alveolus is uneven, rough and corroded (REGÖLY—MÉREI, 1962). It occurs comparatively frequently in our material (24 individuals), that may be a consequence of the large number of caries cases.



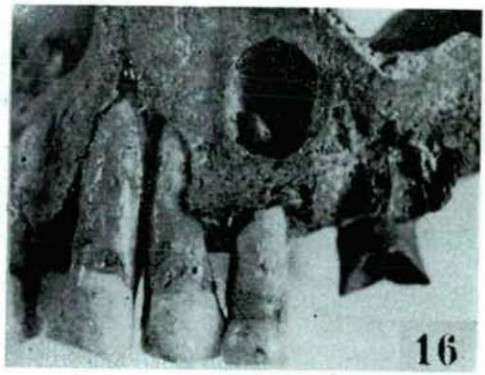




Fig. 15. Hódmezővásárhely—State Farm, Bronze Age, No. 5284, Grave 1. — Hyperodontia ($M_4$) on the right maxilla.

Fig. 16. Szőreg-C, middle Bronze Age, No. 254, Grave 138. — Cyst at left upper $P_1$.

Persisting milk tooth (44) was observed in one case, in the area between $P_1$ and $P_2$ on a female mandible.

The alveolar edge is strongly rarefied at the front teeth on the mandible of find no. 219 from the Szőreg-C site; on this part the roots have become visible (45).

## References

ACSÁDI, GY. — HARSÁNYI, L. — NEMESKÉRI J. (1962): The Population of Zalavár in the Middle Ages. — Acta Arch. Hung. *14*, 113—141.

ANGEL, J. L. (1966): Porotic hyperostosis, Anemias Malarias and Marshes in the Prehistoric Eastern Mediterranean. — Science *153*, 760—762.

BALOGH, K. (1958): Fogászat (Dentistry). — Medicina, Budapest.

BANNER, J. (1933—34): Ásatás a hódmezővásárhelyi Kotacparton (Ausgrabung am Kotacpart bei Hódmezővásárhely.) — Dolgozatok *9—10*, 54—84.

BANNER, J. (1935): Ásatás a hódmezővásárhelyi Kotacparton (Ausgrabungen am Kotacpart bei Hódmezővásárhely.) — Dolgozatok *11*, 97—125.

BANNER, J. (1940): A Régiségtudományi Intézet ásatásai 1940-ben. Újabb ásatás a hódmezővásárhelyi Kökénydombon (Ausgrabungen des Archäologischen Instituts im Jahre 1940. Eine neuere Ausgrabung in Hódmezővásárhely—Kökénydomb.) — Dolgozatok *16*, 181—182.

BANNER, J. (1951): A harmadik ásatás a hódmezővásárhelyi Kökénydombon (The third excavation on the Kökénydomb at Hódmezővásárhely.) — Arch. Ért. *78*, 27—36.

BARTUCZ, L. (1966): Palaeopathologia III. A prehistorikus trepanáció és orvostörténeti vonatkozású sírleletek (Palaeopathology III. Prehistoric trepanation and grave-finds with a medical-historical aspect.) — Publication of the National Medical-Historical Library, Budapest.

BRABANT, H. — NEMESKÉRI, J. (1963): Étude anthropologique et stomatologique d'une série de crânes d'âge Hunnique découverts à Mözs (Hongrie). — Bull. Groupement Int. pour la Recherzhe Scient. en Stomatol. Octobre 1963. No. 4. 1—22.

BROTHWELL, D. R. (1959): The use of non-metrical characters of the skull in differentiating populations. — Bericht über die 6. Tagung der Deutschen Gesellschaft für Anthropologie in Kiel 30.7. bis 2.8.1958. — Homo (Suppl.). 103—109.

BROTHWELL, D. R. (1965): Digging up Bones. — London.

CHARWART, J. — PACOVSKY, J. V. — DUBOVSKY, J. (1964): Beiträge zum Problem der diffusen Knochenerkrankungen. — Wiener Z. inn. Med. *45*, 81—93.

CSALOG, Zs (1963): Archaeologische Forschungen im Jahre 1962. — Rákóczifalva—Kastélydomb. — Arc. Ért. *90*, 298.

CULE, J. — EVANS, L. (1968): Porotic hyperostosis and the Gelligaer skull. — J. Clin. Path. *21*, 753—758.

ENDES, P. (1972): Pathologia I—II. — Medicina, Budapest.

ESSEN—MÖLLER, E. (1928): Statistische Untersuchungen über die persistierende Stirnnaht (Metopismus). — Anthrop. Anz. *5*, 321—326.

FARKAS, GY. (1972): Antropológiai praktikum I. Paleoantropológia metodikák (Anthropological practice I. Palaeoanthropological methods.) (With the collaboration of LENGYEL, I. and B. MARCSIK, A. ). — Attila József University Science Faculty Notes. Szeged.

FOLTINY, I. (1941): Koraréz- és bronzkori temető Deszken (Torontál m.) (Frühkupferzeitliches und bronzezeitliches Gräberfeld in Deszk (Komitat Torontál)). — Folia Arch. *3—4*, 69—98.

FOLTINY, I. (1941a): A szőregi bronzkori temető (Das bronzezeitliche Gräberfeld in Szőreg.) — Dolgozatok *17*, 1—88.

FOLTINY, I. (1942): A "Deszk-F" bronzkori temető (Das bronzezeitliche Gräberfeld "Deszk-F"). — Publications of the Szeged Town Museum *2*, 9—30.

GAZDAPUSZTAI, GY. (1963): Archaeologische Forschungen im Jahre 1962. — Nádudvar—LPG Vörös Csillag. — Arch. Ért. *90*, 298.

GAZDAPUSZTAI, GY. (1963a): Késŏneolitkori telep és temető Hódmezővásárhely—Gorzsán (Siedlung und Friedhof aus dem Spätneolithikum in Hódmezővásárhely—Gorzsa). — Ferenc Móra Museum Yearbook 1963. Szeged. 21—48.

HAMPERL, H. — WEISS, P. (1955): Über die spongiöse Hyperostose an Schädeln aus Alt-Peru. — Virchows Archiv. *327*, 629—642.

HARAGHY, L. (1966): A kórbonctan elemei I—III (The elements of pathology I—III). — Medicina, Budapest.

HEGEDŰS, K. (1973): Archaeologische Forschungen im Jahre 1972. — Vésztő-Mágori halom. — Arch. Ért. *100*, 261.

HENSCHEN, F. (1961): Cribra cranii, a skull condition said to be of racial or geographical nature. — 7th Conf. Int. Soc. Geogr. Path. London. 1960. — Path. et Microbiol. (Basel) *24*, 724.

HENGEN, O. P. (1971): Cribra orbitalia: Pathogenesis and probable etiology. — Homo *22*, 57—76.

HESS, L. (1946): Ossicula wormiana. — Human Biol. *18*, 61—80.

JANSSENS, P. A. (1970): Palaeopathology. Diseases and injuries of prehistoric man. — London.

KENNETH, A. B. (1965): The Etiology and Genetics of Wormian Bones. — Am. J. Phys. Anthr. *23*, 255—261.

KISS, F. (Ed.) (1953): Rendszeres bonctan. Egyetemi tankönyv (Systematic anatomy. University textbook). — Egészségügyi Kiadó, Budapest.

KŐHEGYI, M. (1961): Archaeologische Forschungen im Jahre 1960. — Baja, Dózsa György út 233. — Arch. Ért. *88*, 292.

B. KUTZIÁN, I. (1961): Archaeologische Forschungen im Jahre 1960. — Tiszasziget — Arch. Ért. *88*, 285.

B. KUTZIÁN, I. (1972): The early copper age Tiszapolgár culture in the Carpathian Basin. — Arch. Hung. *48*, 22—406.

LIPTÁK, P. (1962): Homo sapiens — species collectiva. — Anthrop. Közlem. *6*, 17—27.

LIPTÁK, P. (1965): On the taxonomic method in palaeoanthropology (historical anthropology). — Acta Biol. Szeged *11*, 169—183.

LIPTÁK, P. (1971): Embertan és emberszármazástan. Egyetemi tankönyv (Anthropology and human origins. University textbook). — Tankönyvkiadó. 2nd Edition. Budapest.

MARTIN, R. — SALLER, K. (1957—1966): Lehrbuch der Anthropologie. Bd. I—II. — Stuttgart.

NATHAN, H. — HAAS, N. (1966): "Cribra orbitalia". A bone condition of the orbit of unknown nature. — Israel J. Med. Sciences *2*, 171—191.

NEMESKÉRI, J. — HARSÁNYI, L. (1959): Die Bedeutung paläopathologischer Untersuchungen für die historische Anthropologie. — Homo *10*, 203—226.
NEMESKÉRI, J. — HARSÁNYI, L. — GERENCSÉR, GY. (1973): Die biologische Rekonstruktion der Population von Növenthien Kreis Uelzen, aus dem 12—13. Jahrhundert. — Neue Ausgrabungen und Forschungen in Niedersachsen. — Hildesheim. *8*, 127—166.
PATAY, P. (1943): Szentesvidéki rézkori temetők (Kupferzeitliche Gräberfelder aus der Gegend von Szentes). — Arch. Ért. *4*, 26—40.
PÁRDUCZ, M. (1940): Bronz-, szkita-, La Tène és germánkori temető Hódmezővásárhely—Kishomokon (Ein Gräberfeld in Hódmezővásárhely—Kishomok aus der Bronze-, Skythen-La Téne und Germanenzeit). — Dolgozatok *16*, 79—99.
PÁRDUCZ, M. (1942): Preszkita sírok Lebőn (Preskythische Gräber in Lebő). — Dolgozatok *18*, 150—152.
PRATOP, C. D. (1969): Bilateral Parietal Thinning in Bronze Age Skull. — Brit. Med. J. *1*, 55.
RATKÓCZY, N. (1969): Radiologia. — Medicina, Budapest.
REGÖLY—MÉREI, GY. (1962): Palaeopathologia II. Az ősemberi és későbbi emberi maradványok rendszeres kórbonctana (Palaeopathology II. Systematic anatomy of prehistoric and later human remains). — Medicina, Budapest.
TAPPEN, N. C. (1973): Structure of Bone in the Skulls of Neanderthal Fossils. — Am. J. Phys. Anthr. *38*, 93—97.
TÓTH, G. (1942): Újabb ásatás az őszentiváni VIII. lelőhelyen (Neuere Ausgrabungen im VIII. Fundort von Őszentiván). — Dolgozatok *18*, 143—145.
TÓTH, K. (1970): The epidermiology of dental caries in Hungary. — Akadémiai Kiadó, Budapest.
TÓTH, K. — SONKODI, I. (1972): Tápé lelőhelyről származó bronzkori koponyák fogazati állapota (Über den Gebiss-Zustand der Schädel aus dem Bronzezeitalter). — Fogorv. Szemle *65*, 257—264.
TULSI, R. S. (1972): Vertebral column of the Australian aborigine: selected morphological and metrical features. — Z. Morph. Anthrop. *64*, 117—144.
VYHNÁNEK, L. (1971): Analyse der pathologischen Knochenbefunde aus der slawischen Begräbnisstätte von Bilina. — Anthropologie *9*, 129—135.
VYHNÁNEK, L. (1972): Die Blockwirbel in archäologisch geborenem Skelettmaterial. — Anthrop. Anz. *33*, 258—266.
WADSWORTH SCHWARTZ, CH. (1941): Leptomeningeal cysts. From a roentgenological viewpoint. — Am. J. Roent. *46*, 160—165.
WELCKER, H. (1888): Cribra orbitalia, ein ethnologisch-diagnostisches Merkmal am Schädel mehrerer Menschenrassen. — Arch. f. Anthrop. *17*, 1.
WILLIAMS, G. D. — RITSCHIE, W. A. — TERRINGTON, P. F. (1941): Multiple Boney Lesions Sugesting Myeloma in Pre-Columbian Indian aged ten years. — Am. J. Roent. *46*, 351.
ZALOTAY, E. (1933—34): A Szentes-kistőkei rézkori temető (Der Friedhof in Szentes-Kistőke aus der Kupferzeit). — Dolgozatok *9—10*, 85—92.

Address of the authors:
Dr. GY. FARKAS
Dr. ANTÓNIA MARCSIK
Department of Anthropology,
A. J. University, H—6701 Szeged,
P. O. Box 428, Hungary

Acta Biologica Szeged. *21* (1—4), pp. 165—179 (1975)

# SKELETAL REMAINS OF THE AVAR PERIOD AND 10TH CENTURY CEMETERY EXCAVATED AT RÁKÓCZIFALVA—KASTÉLYDOMB

P. LIPTÁK and ANTÓNIA MARCSIK

*Department of Anthropology, Attila József University, Szeged*

(Received October 15, 1974)

## Abstract

72 Avar period and 9 early-Arpadian Age graves were excavated in the area of Rákóczifalva—Kastélydomb in 1962. Altogether 67 skeletal remains were found, in a worse-average state of preservation. The males are generally brachycranic, but the cranical index of the females is more variable. Their braincase (cranial vault) is orthocranic and tapeino-metriocranic, and the forehead is stenometopic. In the case of both sexes the face is generally mesoprosopic, mesene. The fossa canina is absent or shallow. The orbits are usually hypsiconch, and the nasal index is mesorrhine (in the females chamaerrhine). The stature is very variable. The number of anthropological finds suitable for taxonomic analysis is only twelve, of which nine individuals are of Europid character. Within these, the Cromagnoids (crC) predominate, with various proportions of the Mongolid racial component. Three resp. four individuals could be classified as Mongolids. In one case the Baikal race (b) could be diagnosed and in another the Saianic (sa), while for two individuals the Mongolid component could not be determined in detail. In the nine early Arpadian-Age graves only two skeletons are in a good state of preservation; one of them shows Mediterranean, and the other one Mongolid character. The Avar period and 10th century cemetery at Rákóczifalva—Kastélydomb is important as representing original Avars who immigrated here from the central regions of Asia, while in addition the presence of the Mongolid element in the 10th century is evidence of the survival of the Avar population.

## Introduction

Excavations were carried out by Zsolt Csalog in the area called Rákóczifalva—Kastélydomb, not far from Szolnok, in May and June 1962. He uncovered altogether 178 graves, including early and late Bronze-Age and Gepid graves, 72 Avar-period and nine early Arpadian-Age (10th century) graves (CSALOG, 1963). The archaeological elaboration of the Avar period graves is being performed by László Selmeczi, archaeologist of the Museum in Szolnok; special thanks are due to him for placing his manuscript at our disposal. The material of 64 graves has passed into the collection of the Department of Anthropology at Attila József University. In three cases there were double burials (graves 32, 38 and 124), and therefore the number of finds to be elaborated has risen to 67. 12 of the 43 crania are in a good state of preservation (i.e. measurable), and 31 are fragmentary (Table 1). The great majority of crania are associated with skeletons (38 cases), but there are crania without postcranial skeletons (5 cases). It appears from the data of Table 1 that the preservation of the Avar period skeletons unearthed in the area of Rákóczifalva—Kastélydomb is worse than the average.

*Table 1.* Rákóczifalva-Kastélydomb, Avar period, skeletal material.

| Characterization of the material | | In. I. | Inf. II. | Juv. | Ad. | Mat. | Sen. | Total |
|---|---|---|---|---|---|---|---|---|
| Fragmentary (unmeasured) | Males ..... | — | — | — | 2 | 8 | 1 | 11 |
| | Females ..... | — | — | 1 | 6 | 6 | — | 13 |
| | Undeterminable..... | 1 | 6 | — | — | — | — | 7 |
| | Total: | 1 | 6 | 1 | 8 | 14 | 1 | 31 |
| Well preserved (measured) | Males ..... | — | — | — | 1 | 6 | — | 7 |
| | Females ..... | — | — | — | 3 | 2 | — | 5 |
| | Total: | — | — | — | 4 | 8 | — | 12 |
| | Sum-total: | 1 | 6 | 1 | 12 | 22 | 1 | 43 |

## General characterization of the Avar-period population

In the elaboration of the skeletal material, we have made use of MARTIN's method (1928). From the results in Tables 2 to 4, a general characterization of the anthropological material of the Avar-period cemetery at Rákóczifalva—Kastélydomb, relating to both sexes, can be given in the following way; owing to the low number of cases, no parameters have been calculated.

The crania of the males exhibit a brachycranic tendency, while in the case of the females lower and higher values than this occur as well. The cranial profile in the vertical norm is ovoid and pentagonoid, respectively. The braincase is generally orthocranic and tapeino-metriocranic, respectively. The protuberantia occipitalis externa is of degrees 0 and 1; the mastoid process of the males is of medium size, and that of the females is small. On the basis of the transversal-frontoparietal index the cranium is stenometopic and metriometopic; while in the case of the males the forehead slopes somewhat, in the females it is steep. The glabella is of degrees 1 and 2, respectively. Taking into consideration the cranial capacity values, the males are aristencephalic, and the females euencephalic but, due to their fragmentary state, only in two cases each. The face of the males is usually mesoprosopic, mesene; that of the females is similar, but hyperleptoprosopy also occurs. The fossa canina is absent or shallow. In both sexes the orbits are hypsiconch, being a little square or rounded. As regards of the value of the nasal index, mesorrhiny is more characteristic of the males, and chamaerrhiny of the females. The nasal spine is generally missing, but if found it may show almost every degree. The facial angle profile is uniformly orthognathous, alveolar prognathism manifesting itself only rarely. The stature is very variable for both sexes; a tall stature is comparatively frequent in both males and females (Table 4).

Sutura metopica occurs in two cases (graves 19 and 154) and lambdoid sutural bones (grave 92) and os apicis (grave 124) in one case each. Individual measurements and indices are to be found in Tables 2 and 3, and the measurements of long bones in Table 4.

*Table 2.* Rákóczifalva-Kastélydomb, Avar period; measurements, indices and morphologic characters. — Males (1).

| No. of measurements (Martin) | Measurements and indices | 7. 3519 Ad. | 28. 2532 Mat. |
|---|---|---|---|
| 1. | Glabello-occipital length | 177 | — |
| 1c. | Metopion-occipital length | 176 | — |
| 5. | Basion-nasion length | — | — |
| 8. | Maximum breadth of cranium | 137 | — |
| 9. | Minimum frontal breadth | 93 | 90 |
| 17. | Basion-bregma height | — | — |
| 20. | Porion-bregma height | 117 | — |
| 32/1-a | Frontal angle | 49° | — |
| 38. | Calculated cranial capacity | — | — |
| 40. | Basion-prosthion length | — | — |
| 45. | Bizygomatic breadth | — | 135 |
| 46. | Maxillar breadth | 101 | 95 |
| 47. | Total facial height | (123) | (119) |
| 48. | Upper facial height | 72 | 70 |
| 51. | Orbital breadth | 38 | 36 |
| 52. | Orbital height | 31 | 33 |
| 54. | Nasal breadth | 26 | 27 |
| 55. | Nasal height | 51 | 52 |
| 62. | Palatal length | 42 | 48 |
| 63. | Palatal breadth | 37 | 35 |
| 65. | Bicondylar-diameter | 128 | — |
| 66. | Bigonial-diameter | 103 | — |
| 69. | Mental height | 37 | 37 |
| 70. | Ramus height | 75 | 66 |
| 71. | Ramus breadth | 29 | 29 |
| 72. | Total facial angle | 85° | — |
| 8:1 | Cranial index | 77,4 | — |
| 17:1 | Length-height index | — | — |
| 17:8 | Breadth-height index | — | — |
| 9:8 | Transvers.-frontopar. index | 67,9 | — |
| 47:45 | Facial index | — | (88,2) |
| 48:45 | Upper facial index | — | 51,8 |
| 52:51 | Orbital index | 81,6 | 91,7 |
| 54:55 | Nasal index | 50,9 | 51,9 |
| 63:62 | Palatal index | 88,1 | 72,9 |
| Vertical norm | | Pent. | — |
| Glabella | | 3 | 3 |
| Protuberantia occipitalis externa | | 1 | — |
| Fossa canina | | 3 | 2 |
| Spina nasalis anterior | | — | 4 |
| Prognathia alveolaris | | 2 | 1 |
| Calculated stature | | 160 | 166 |
| Taxon | | m-moid | (moid) |

Males (2).

| 50.<br>3553<br>Mat. | 54.<br>3556<br>Mat. | 91.<br>3581<br>Mat. | 92.<br>3582<br>Mat. | 154.<br>3630<br>Mat. | No. of<br>measurements<br>(Martin) |
|---|---|---|---|---|---|
| — | 177 | — | 172 | 180 | 1. |
| — | 174 | — | 166 | 178 | 1c. |
| — | 104 | — | — | 98 | 5. |
| 145 | 146 | — | 147 | 150 | 8. |
| 84 | 103 | 100 | 103 | 100 | 9. |
| — | 143 | — | — | 129 | 17. |
| 115 | 120 | — | 117 | 110 | 20. |
| 53° | 53° | — | 50° | 50° | 32/1-a. |
| — | 1503 | — | — | 1449 | 38' |
| — | 102 | — | — | 98 | 40. |
| 147 | (143) | 146 | (144) | 141 | 45. |
| 102 | 103 | 103 | 101 | 109 | 46. |
| 128 | 126 | — | 118 | — | 47. |
| 76 | 78 | 75 | 75 | 76 | 48. |
| 38 | 43 | 40 | 43 | 39 | 51. |
| 34 | 36 | 34 | 39 | 31 | 52. |
| 25 | 28 | 28 | 24 | 27 | 54. |
| 56 | 54 | 55 | 54 | 57 | 55. |
| 48 | 52 | — | 48 | 46 | 62. |
| 43 | 41 | — | 42 | — | 63. |
| — | 132 | — | — | — | 65. |
| — | 111 | 132 | 118 | — | 66. |
| 38 | 34 | — | 31 | 37 | 69. |
| 66 | 76 | — | 73 | 68 | 70. |
| 29 | 34 | — | 27 | 36 | 71. |
| 90° | 85° | — | 85° | 86° | 72. |
| — | 82,5 | — | 85.5 | 83,3 | 8:1 |
| — | 80,8 | — | — | 71,7 | 17:1 |
| — | 97,9 | — | — | 86,0 | 17:8 |
| 57,9 | 70,5 | — | 70,1 | 66,7 | 9:8 |
| 87,1 | (88,1) | — | (81,9) | — | 47:45 |
| 52,5 | 55,2 | 51,4 | 52,1 | 53,9 | 48:45 |
| 89,5 | 83,7 | 85,0 | 90,7 | 79,5 | 52:51 |
| 44,6 | 51,8 | 50,9 | 44,4 | 47,4 | 54:55 |
| 89,6 | 78,8 | — | 87,5 | — | 63:62 |
| Ovoid | Ovoid | — | Ovoid | Ovoid | Vert. norm. |
| 4 | 3 | 3 | 4 | 3 | Glabella |
| — | 0 | — | 0 | 0 | Prot. occ. ext. |
| 2 | 3 | 2 | 2 | 2 | Fossa canina |
| — | 3 | — | — | — | Spina n. ant. |
| 1 | 2 | 1 | 1 | 3 | Progn. alv. |
| 170 | — | — | 170 | — | Stature |
| crC-moid | crC-moid | crC-x | crC-moid | crC-moid | Taxon |

*Table 3.* Rákóczifalva-Kastélydomb, Avar period; measurements, indices and morphologic characters. — Females.

| No. of measurement (Martin) | 19. 3225 Ad. | 33. 3538 Ad. | 35. 3540 Mat. | 78. 3570 Mat. | 124. 3610 Ad. |
|---|---|---|---|---|---|
| 1. | — | 175 | 179 | 176 | 166 |
| 10. | — | 169 | 175 | 175 | 161 |
| 5. | — | 93 | — | 93 | — |
| 8. | (143) | (140) | 131 | 135 | 142 |
| 9. | 97 | 90 | 92 | 95 | 90 |
| 17. | — | 127 | — | 126 | — |
| 20. | 106 | — | 110 | 110 | 115 |
| 32/1-a. | 50° | 48° | 50° | 43° | 55° |
| 38. | — | 1275 | — | 1222 | — |
| 40. | — | 95 | — | 90 | — |
| 45. | 139 | — | (117) | 122 | 134 |
| 46. | 107 | 97 | 93 | 92 | 101 |
| 47. | 121 | 115 | (114) | — | 114 |
| 48. | 71 | 69 | 66 | 77 | 67 |
| 51. | 42 | 39 | 34 | 39 | 39 |
| 52. | 34 | 34 | 31 | 34 | 32 |
| 54. | 27 | 25 | 23 | 26 | 27 |
| 55. | 49 | 48 | 45 | 54 | 51 |
| 62. | 44 | 44 | 45 | 45 | 48 |
| 63. | 41 | 39 | — | 35 | 34 |
| 65. | 120 | — | — | — | — |
| 66. | 100 | 101 | — | — | 93 |
| 69. | 32 | 32 | 35 | 32 | 31 |
| 70. | 62 | 64 | 63 | 71 | 66 |
| 71. | 33 | 31 | 32 | 28 | 34 |
| 72. | 90° | 88° | 90° | 88° | 86° |
| 8:1 | — | 80.0 | 73.2 | 76.7 | 85.5 |
| 17:1 | — | 72.6 | — | 71.6 | — |
| 17:8 | — | 90,7 | — | 93.3 | — |
| 9:8 | 67.8 | 64.3 | 70.2 | 70.4 | 63.4 |
| 47:45 | 87.1 | — | 97.4 | — | 85.1 |
| 48:45 | 51.1 | — | 56.4 | 63.1 | 50.0 |
| 52:51 | 80.9 | 87.2 | 91.2 | 87.2 | 82.1 |
| 54:55 | 55.1 | 58.3 | 51.1 | 48.1 | 52.9 |
| 63:62 | 93.2 | 88.6 | — | 77.8 | 77.1 |
| Vert. norm. | — | Ovoid | Pent. | Pent. | Ovoid |
| Glabella | 2 | 1 | 2 | 1 | 1 |
| Prot. occ. ext. | — | 0 | — | 0 | 2 |
| Fossa canina | 1 | 2 | 1 | 1 | 2 |
| Spina n. ant. | 2 | 2 | — | — | 3 |
| Prog alv. | 1 | 2 | 1 | 2 | 1 |
| Stature | 159 | 155 | — | 159 | 160 |
| Taxon | sa | br-moid | b—x | m—x | p-moid |

*Table 4.* Rákóczfalva-Kastélydomb, measurements of long bones and the calculated stature.

| No. of grave | No. of inventory | Sex | Femur | | | | Tibia | | Humerus | | Radius | | Statu-re |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Max. length | | Max. lenght in nat. position | | | | | | | | |
| | | | right | left | right | left | righ | left | righ | left | righ | left | |
| Avar period | | | | | | | | | | | | | |
| 7. | 3519 | ♂ | 432 | 430 | 430 | 426 | — | — | — | 317 | — | — | 160 |
| 19. | 3525 | ♀ | 423 | 423 | 420 | 420 | — | — | 304 | 295 | 225 | 230 | 159 |
| 28. | 3532 | ♂ | 450 | 450 | 448 | 441 | 370 | 372 | — | 310 | 248 | — | 166 |
| 32/a. | 3536 | ♀ | — | 460 | — | 455 | — | — | 322 | 318 | — | — | 161 |
| 33. | 3538 | ♀ | 408 | 410 | 405 | 408 | 345 | 343 | 298 | — | — | — | 155 |
| 50. | 3553 | ♂ | 463 | 465 | 450 | 460 | 390 | 387 | 335 | 334 | 262 | 260 | 170 |
| 68. | 3564 | ♂ | 465 | 465 | 460 | 460 | 394 | 394 | — | — | — | — | 171 |
| 78. | 3570 | ♀ | 443 | 445 | 438 | 438 | 355 | 355 | 320 | 315 | 235 | — | 159 |
| 92. | 3582 | ♂ | 482 | 487 | 480 | 482 | — | 390 | 335 | — | 253 | 253 | 170 |
| 111. | 3600 | ♂ | 415 | 415 | 410 | 410 | 363 | 360 | — | — | — | — | 162 |
| 118. | 3604 | ♂ | 442 | 445 | 438 | 440 | — | — | — | 325 | 250 | 250 | 166 |
| 124. | 3610 | ♀ | 410 | 413 | 408 | 409 | 348 | 350 | 305 | 298 | 226 | 223 | 160 |
| 10th century | | | | | | | | | | | | | |
| 20. | 3526 | ♀ | 405 | 405 | 403 | 403 | 340 | 340 | 295 | 288 | 228 | 230 | 154 |
| 25. | 3530 | ♀ | 404 | 408 | 402 | 404 | 330 | 333 | 306 | 306 | 223 | 220 | 154 |

## Taxonomic analysis of the Avar period skeletal remains

As compared to the number of excavated crania and postcranial skeletons, unfortunately only very few of the anthropological finds are in a good state of preservation and suitable for taxonomic analysis: seven males and five females, i.e. twelve individuals altogether. Taxonomic analysis was based on the skeletal systematics of one of the authors (LIPTÁK, 1965; 1971). Nine of the twelve individuals studied are *Europids* but with more or less Mongolid elements. In the group of Europids tall-statured but brachycranic Cromagnoids (crC) predominate; these occurred only among the males (Fig. 1), almost without exception with a certain proportion of some Mongolid racial component. The proportion of the gracile Mediterranean component (m) is subordinate in both sexes, as are those of the Pamirian (p) and the undetermined brachycranic components in the females (Figs. 2 and 3).

Three (resp. four) individuals could be classifield in the group Mongolids. On the basis of the craniosystematics already well-elaborated for the Avars in Hungarian territory (LIPTÁK, 1957; 1959), in one of the cases (grave 35) the dolichocranic protomongolid Baikal (b) race, could be recognized, and in the other case (grave 19) the low-faced Mongolid race. In the Soviet anthropological literature, the latter has been named "Katanga type", but since this is also a river name in Africa, it

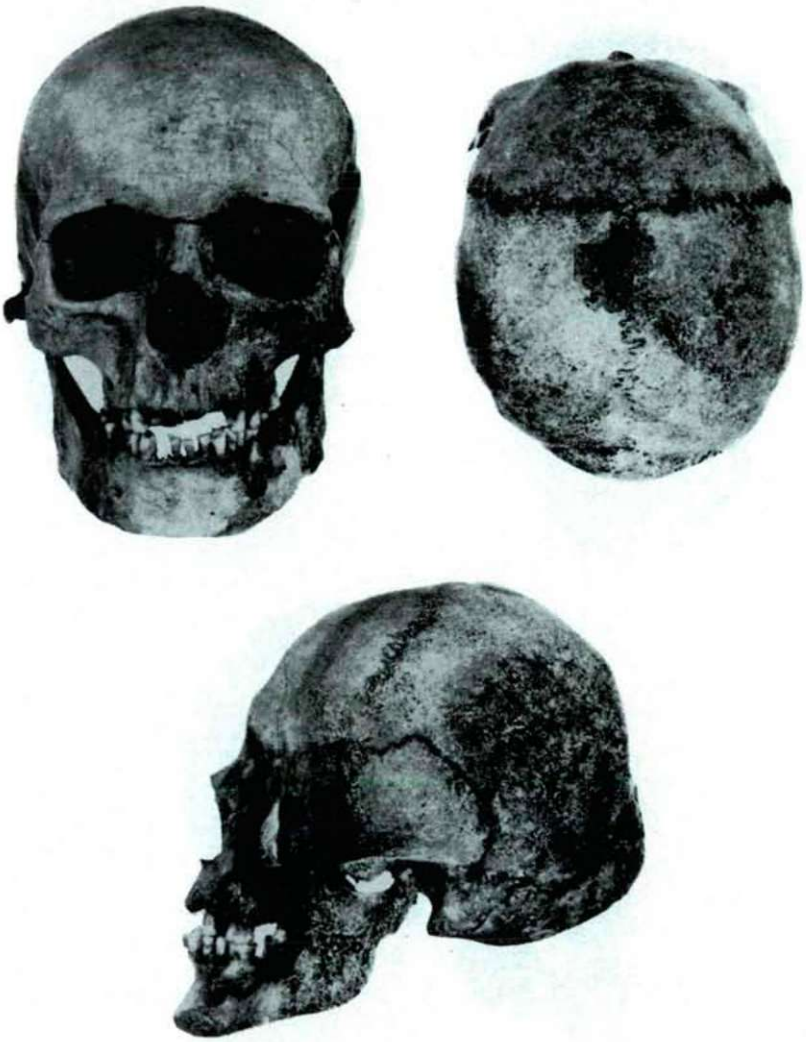


Fig. 1. Rákóczifalva—Kastélydomb, Avar period, grave 54 (3556), male crC-moid.

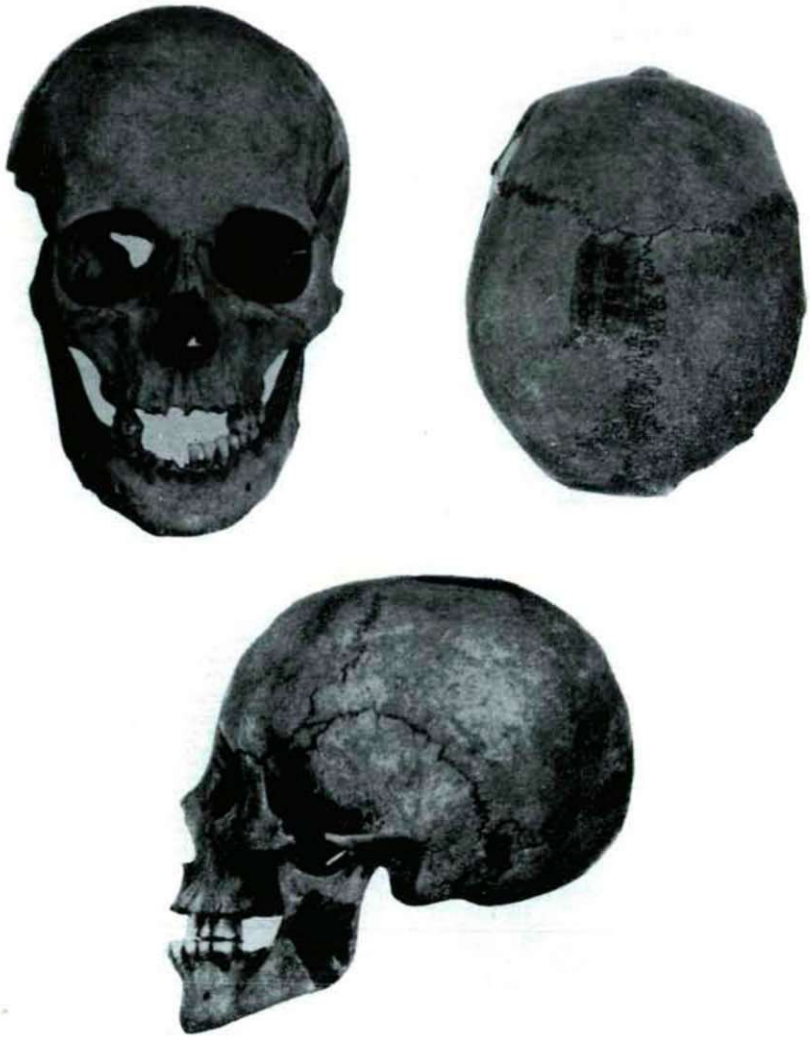


Fig. 2. Rákóczifalva—Kastélydomb, Avar period, grave 33 (3538), female, br-moid.

Fig. 3. Rákóczifalva—Kastélydomb, Avar period, grave 124 (3610), female, p-x(moid).

*Table 5*. Comparison of some series of the Avar period.

| Place of excavation | Author date of publication | Number of suitable for metrical analysis | Distrbution of cranial index | | | | Major taxons |
|---|---|---|---|---|---|---|---|
| | | | 70—75 | 75—80 | 80—85 | 85—x | |
| Áporkai-Ürbőpuszta | Lipták 1951 | Male: 7<br>Female: 6 | —<br>— | 2<br>4 | 4<br>4 | —<br>— | brachycephals, nordoid, cromagnoid |
| Kiskőrös-Vágóhíd | Nemeskéri 1955 | Male: 7<br>Female: 2 | —<br>— | 1<br>— | 4<br>2 | —<br>— | saianic, central-asiatic |
| Kiskőrös-Pohibujmackó | Lipták 1956 | Male: 7<br>Female: 3 | —<br>— | 3<br>2 | 3<br>1 | —<br>— | cromagnoid, brachycephals, mongoloid |
| Rákóczifalva-Kastélydomb | — | Male: 7<br>Female: 5 | —<br>1 | 1<br>1 | 2<br>1 | 1<br>1 | cromagnoid, brachycephals, saianic, bajkal |

may give rise to misunderstanding. In Asia, this anthropological component is frequent among the populations living in the Saian-mountains: the Saianic Mongolid component has been mentioned by Soviet anthropologists; accordingly, instead of the designation "Tungid type", which is wide-spread in the Hungarian anthropological literature, the name Saianic (sa) race seems advisable (Fig. 4) because of the fragmentary condition of the cranium it is not oriented in the Frankfurt horizontal plane. In a single case (grave 28) the Mongolid character is unquestionable but, due to the defective cranium, a more exact determination is not possible. There was a Mongolid cranium in the fragmentary, i.e. unmeasured material, but this was not determined more closely (grave 39). Both above mentioned racial components are characteristic of the Avar period cemeteries in Hungary.

The number of crania in a good state of preservation being only twelve, this small series is hardly suitable for drawing far-reaching conclusions. The cemetery is important anthropologically, as the material in a good state of preservation clearly indicates its definite Mongolid or Europo-Mongolid character. This cemetery not only stems from the Avar-period but also involves Avars immigrating to the Carpathian Basin from the inner regions of Asia.

A segregation of groups within the cemetery is not possible on the basis of the anthropological material. A comparison with other Avar-period cemeteries such as those at Ürbőpuszta (LIPTÁK, 1951), Kiskőrös—Vágóhíd (NEMESKÉRI, 1955) and Kiskőrös—Pohibujmackó (LIPTÁK, 1956) — is made possible by Table 5.

Apart from the 72 Avar-period graves, nine early Arpadian-Age (10th century) graves were found as well. From these, two skeletons are in a good state of preservation (Table 6), and seven are fragmentary ones. Taxonomic analysis indicates the individual from grave 20 to be predominantly Mediterranean (Fig. 5), while that from grave 25 shows Mongolid character, not determined more closely (Fig. 6). The presence of the Mongoloid element is proving that the Avar population survived in the 10th century, as has been proved in the case of the cemetery at Szarvas—Kákapuszta—Kettőshalom (LIPTÁK—MARCSIK, 1970).

*Table 6.* Rákóczifalva-Kastélydomb, 10th century; measurements, indices and morphologic characters. — Females.

| No. of measurements (Martin) | Measurements and indices | 20. 3526 Ad. | 25. 3530 Ad. |
|---|---|---|---|
| 1. | Glabello-occipital length | 176 | 170 |
| 1c. | Metopion-occipital length | 177 | 169 |
| 5. | Basion-nasion length | 96 | 92 |
| 8. | Maximum breadth of cranium | 132 | 137 |
| 9. | Minimum frontal breadth | 88 | 94 |
| 17. | Basion-bregma height | 131 | 126 |
| 20. | Porion-bregma height | 110 | 108 |
| 32*f*1-a. | Fontal angle | 48 | 50 |
| 38. | Calculated cranial capacity | 1258 | 1240 |
| 40. | Basion-prosthion length | 92 | 85 |
| 45. | Bizygomatic breadth | 124 | 127 |
| 46. | Maxillar breadth | 93 | 91 |
| 47. | Total facial height | 107 | 112 |
| 48. | Upper facial height | 64 | 71 |
| 51. | Orbital breadth | 39 | 38 |
| 52. | Orbital height | 31 | 33 |
| 54. | Nasal breadth | 22 | 26 |
| 55. | Nasal height | 44 | 50 |
| 62. | Palatal length | 41 | 39 |
| 63. | Palatal breadth | 36 | 34 |
| 65. | Bicondylar-diameter | 119 | 111 |
| 66. | Bigonial-diameter | 96 | 90 |
| 69. | Mental height | 28 | 31 |
| 70. | Ramus height | 65 | 60 |
| 71. | Ramus breadth | 32 | 28 |
| 72. | Total facial angle | 85° | 88° |
| 8:1 | Cranial index | 75,0 | 80,6 |
| 17:1 | Length-height index | 74,4 | 74,1 |
| 17:8 | Breadth-height index | 99,2 | 91,9 |
| 9:8 | Transvers.-frontopar. index | 66,7 | 68,6 |
| 47:45 | Facial index | 86,3 | 88,1 |
| 48:45 | Upper facial index | 51,6 | 55,9 |
| 52:51 | Orbital index | 79,5 | 86,8 |
| 54:55 | Nasal index | 50,0 | 52'0 |
| 63:62 | Palatal index | 87,8 | 87,2 |
| | Vertical norm | Pent. | Ovoid |
| | Glabella | 1 | 2 |
| | Protuberantia occipitalis externa | 0 | 0 |
| | Fossa canina | 2 | 3 |
| | Spina nasalis anterior | 2 | 2 |
| | Prognathia alveolaris | 1 | 2 |
| | Calculated stature | 154 | 154 |
| | Taxon | m-x | moid |

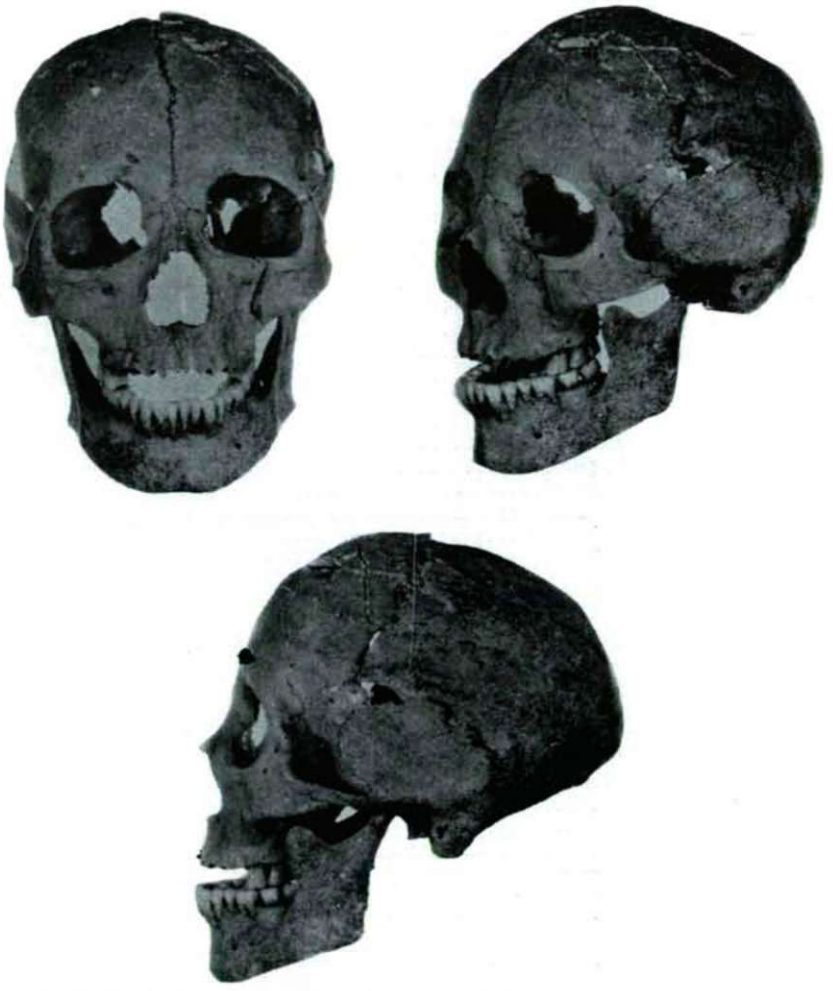


Fig. 4. Rákóczifalva—Kastélydomb, Avar period, grave 19 (3526), female, sa.

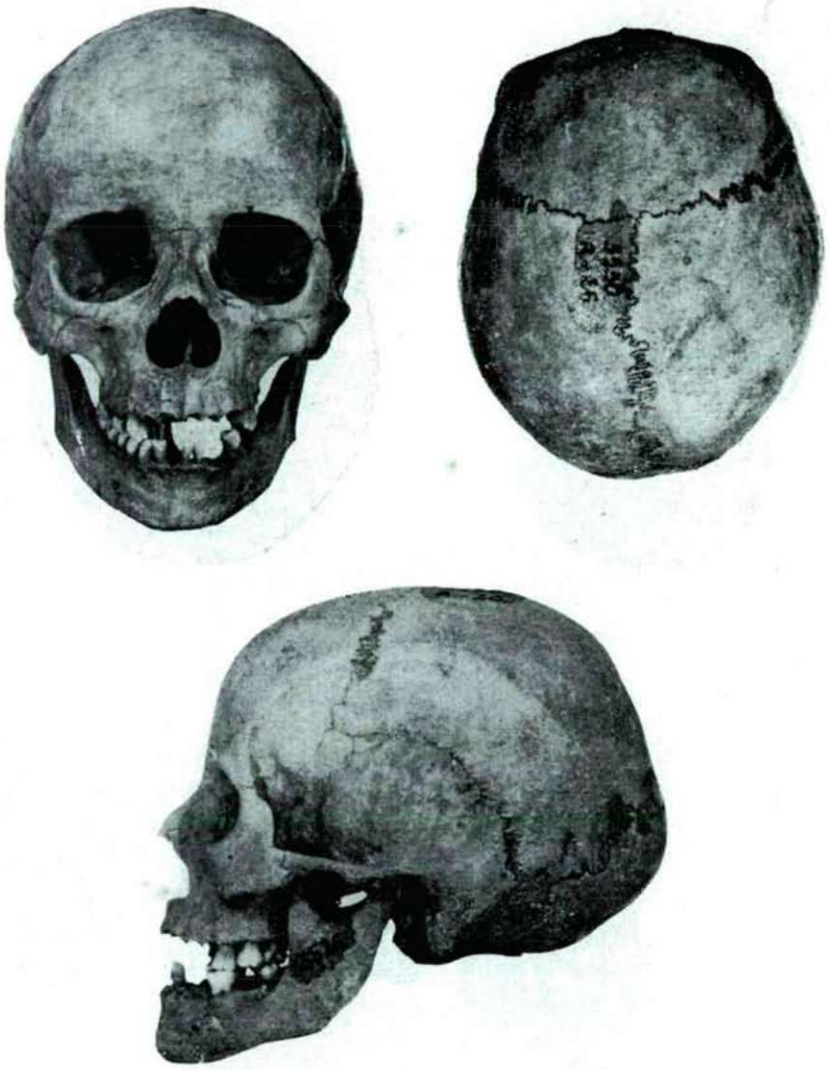


Fig. 5. Rákóczifalva—Kastélydomb, 10th century, grave 20 (3526), female, m-x.

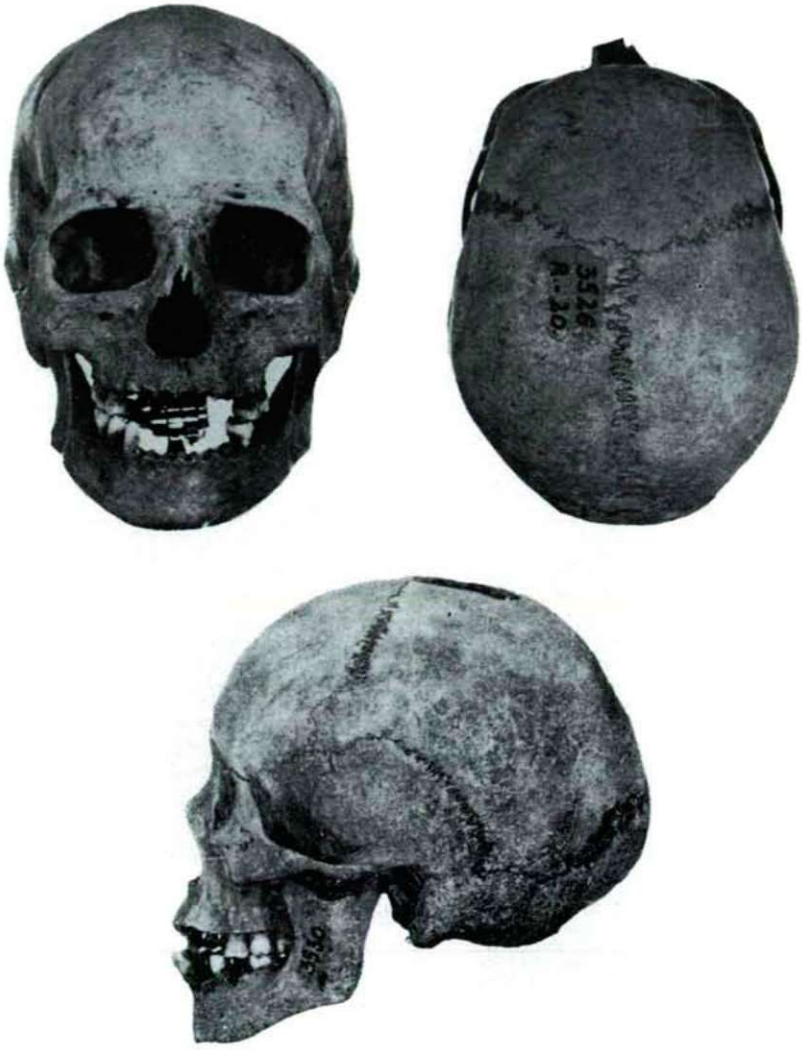



Fig. 6. Rákóczifalva—Kastélydomb, 10th century, grave 25 (3530), female, moid.

## References

CSALOG, Zs. (1963): Rákóczifalva—Kastélydomb (in Archäeologische Forschungen im Jahre 1962).— Arch. Ért. *90*, 298.

LIPTÁK, P. (1951): Étude anthropologique du cimetière avare d'Áporkaiürbőpuszta. — Ann. Hist. — Nat. Mus. Nat. Hung. $I_1$ 232—259.

LIPTÁK, P. (1956): Contributions à l'anthropologie des temps avars de la région de Kiskőrös. — Crania Hung. *1*, 47—52.

LIPTÁK, P. (1957): Awaren und Magyaren im Donau-Theiss Zwischenstromgebiet. — Acta Arch. Hung. *8*, 201—208.

LIPTÁK, P. (1959): The "Avar period" Mongolids in Hungary. — Acta Ach. Hung. *10*, 269—272.

LIPTÁK, P. (1965): On the taxonomic method in paleoanthropology (historical anthoropology). — Acta Biol. Szeged *11*, 169—184.

LIPTÁK, P. (1971): Embertan és emberszármazástan (Anthropology and human origins). 2nd ed. — University texbook, Budapest.

LIPTÁK, P.—MARCSIK, A. (1970): Skelettreste von Szarvas—Kákapuszta—Kettőshalom. Zur Frage der Awarischen—Altungarischen Verbindungen. — MFMÉ. 45—57.

MARTIN, R. (1928): Lehrbuch der Anthropologie. 2nd ed. Jena.

NEMESKÉRI, J. (1955): Étude anthropologique des squelettes du clan princier avar découverts au cimetière de Kiskőrös—Vágóhíd. (in LÁSZLÓ, GY.: Études archéologiques sur l'histoire de de la société des Avars.) — Arch. Hung. *34*, 189—210.

Address of the authors:
Prof. Dr. P. LIPTÁK
Dr. ANTÓNIA MARCSIK
Department of Anthropology,
A. J. University, H—6701 Szeged,
P. O. Box 428, Hungary

Acta Biologica Szeged. *21* (1—4), pp. 181—192 (1975)

# PALAEOPATHOLOGICAL CHARACTERIZATION OF THE SKELETONS OF AN AVAR SERIES (KUNSZÁLLÁS—FÜLÖPJAKAB)

I. VARGA—ANTÓNIA MARCSIK

*Department of Anthropology, Attila József University, Szeged*

(Received July 6, 1974)

## Abstract

At excavating a part of cemetery by Kunszállás—Fülöpjakab (Alkotmány co-operative farm), the skeletons of fifty graves could be preserved. A detailed metrical, morphological and taxonomic analysation of these was carried out already earlier. At the paleopathological evaluation of the series we found the deformation spondylosis deformans in largest numbers, and in smaller numbers osteoporosis, arthrosis deformans, spondylarthrosis, congenital anomalies and, in a single case, block-vertebra. These deformations are limited to the skeletons of ten individuals. Eight of the ten individuals are placed beside one another on the sketch map of cemetery — almost semicircularly. This incidence , as well as the connection according to an earlier blood group determination are showing the eight individuals to belong possibly to the same relation circle. Beside the skeletons showing the most serious deformations, we find a poorer grave furniture — as compared to the average of the cemetery.

## Introduction

From the region of Kunszállás—Fülöpjakab (Alkotmány co-operative farm), ELVIRA H. TÓTH (Museum of Kecskemét) unearthed a part of a late Avar-age cemetery in 1967 and 1970. The graves are rich in furniture: there came to light gold, silver, bronze goods, thong ends, a sword, and animal bones (H. TÓTH, 1968; 1971).

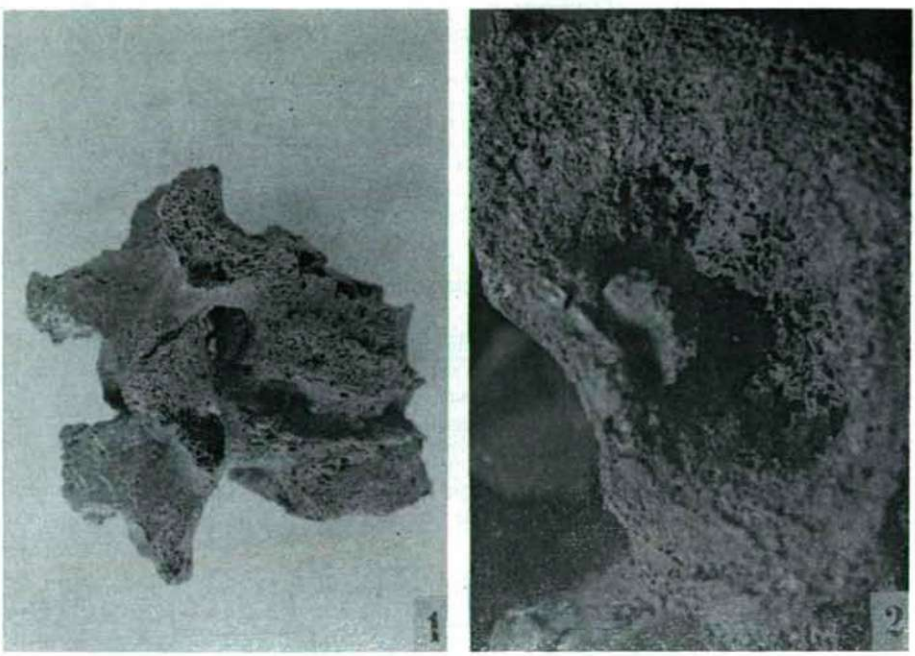
The metrical and morpho-taxonomic elaboration of the data of altogether fifty individuals was carried out by LIPTÁK—VARGA (1974) already earlier, their most important finds are as follows:

31 of the 50 individuals (62 per cent) are in a good state of preservation, nine of these are males, eighteen females while the number of the undeterminable ones is four (Inf. II). On the basis of metrical values and morphological features, the taxonomic analysis is showing the following distribution of the sixteen adults: the Mongolids are predominant, i. e., Central-Asiatic (ca), Baikal (b), and Saianic (sa) ones. There follow then the Europo-Mongolids whose more exact separation was not possible. It is interesting that the Europid great race is only represented by a single case, more exactly a Cromagnoid-B (crB). The authors have described the anatomical variations of the crania in the series (os bregmaticum, sutura metopica, Worm's bones, torus palatinus), and the different morphological features (unusually deep palate, spatulate incisors), as well, that called attention to analyse the part of cemetery pathologically. That investigation was carried out both on the postcranial skeletons and the crania, and in some cases we could study

the morphologically well-distinguishable pathological deformations by means of X-ray pictures. (The X-ray pictures were made in Hospital II of the Town Council, Szeged, and Town Polyclinic for Specialist Consultation, for what we should like to express our gratitude in this way, too).

## Discussion of palaeopathological deformations

Grave 49 (Ad., female): on the rim of the corpora of vertebrae we see a weak osteophyte-formation. Lumbar vertebrae 2 and 3 are worth mentioning: their processus articulares are ossified completely, as well as the middle part of their corpora (Fig. 1). The height of the corpus of lumbar vertebra 3 is smaller than that of vertebra 2, the middle part of the former one is conoidally high and the corpus

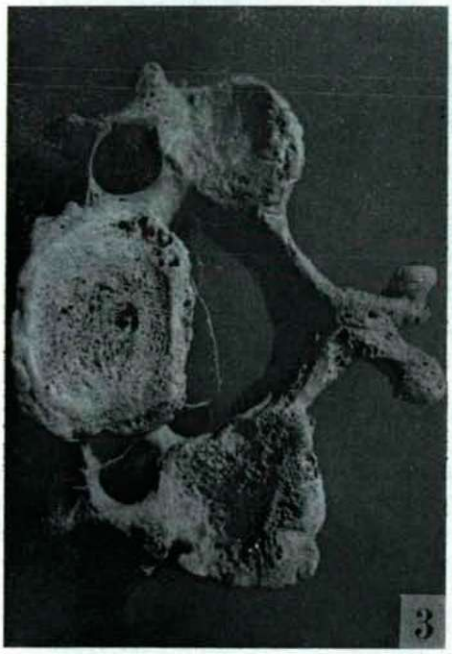


1. Grave 49 (Ad., female) — block vertebra.
2. Grave 49 (Ad., female) — fossalike impression of the corpus of dorsal vertebra 12.

of vertebra 2 is quasi "drawn over that, got wedged in it. In that spinal section, in the corpus of lumbar vertebra 3, on its surface by the vertebral canal, a deep fossalike formation may be observed. A similar formation is to be seen in the last dorsal vertebra, as well (Fig. 2); the difference lies in that, in this case, the deep impression, spoling towards the vertebral foramen, is imbedding a foramen by the

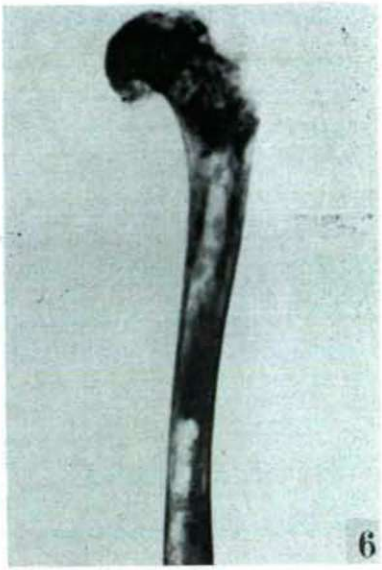
vertabral canal. (This foramen may, of course, have come into being in a postmortal way, as well). In cervical vertebra 3, on the right, the foramen transversarium is divided by a narrow bridge.

— spondylosis deformans;
— partial block;
— congenital anomaly.



3. Grave 7 (Sen., female) — spondylarthrosis.
4. Grave 7 (Sen., female) — osteoporotic vertebra.

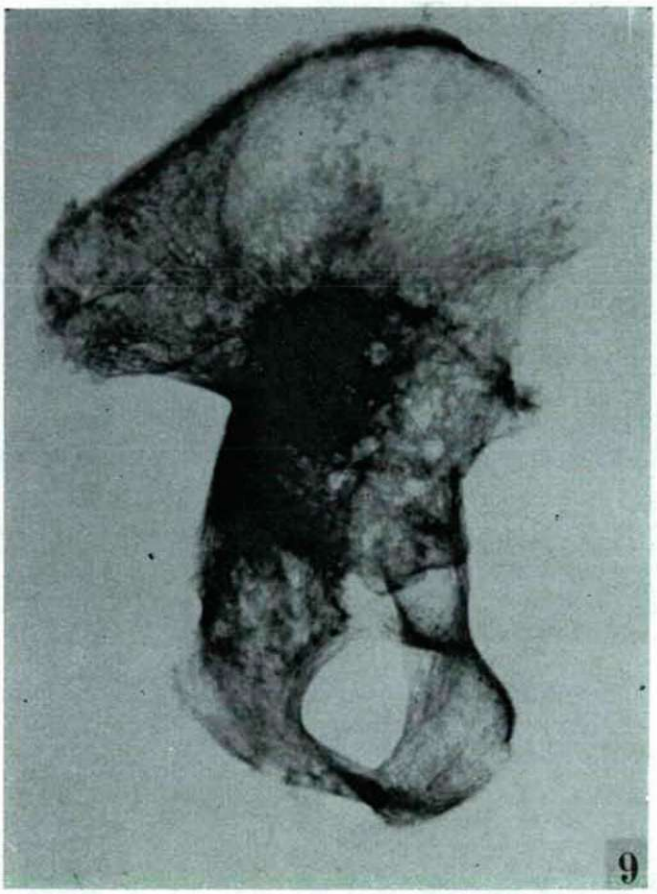
Grave 7 (Sen., female): in the epistropheus, in its left processus articularis inferior, the shape of the articular surfaces is deformed, with small foramina on its surface; the left facies articularis superior et inferior of cervical vertebra 3 and the left facies articularis inferior of cervical vertebra 4 are deformed, on the rims of corpora there is crenation to be observed, and at the surface there are everywhere tiny, smaller or larger foramina (Fig. 3); in the other cervical vertebra there are the deformations described above but in a more serious outward form (Fig. 4); the spongious substance of the corpus of dorsal vertebra 9 is characteristically depressed, with osteophyte-formation on the rim (Fig. 5), below the serrated zone, at the external surface there is longitudinal plication with small foramina found on the surface of processus articularis superior et inferior, as well. In the right femur, the fovea capitis is extensive and foraminiferous, with rarefied fields in its X-ray picture (Fig. 6); in the left femur a serious deformation can be seen: the caput femoris is flattened,



5. Grave 7 (Sen., female) — spondylosis deformans.
6. Grave 7 (Sen., female) — osteoporotic rarefaction in the X-ray photograph of the femur.

reminding of a mushroom-head, and deformed, with a number of smaller or larger foramina on the surface (Fig. 7); the coxa is of vara position, in the left acetabulum, the facies lunata and fossa acetabuli are almost melted with smaller or larger impressions in them, with porous zones (Fig. 8), on the limbus with spongy laying on; there is further porosity in the facies auricularis and rarified zones in the X-ray picture of pelvis (Fig. 9); on the left caput humeri there is a weak crest-formation. The Ca/P quotient of bones is below the average value of all the individuals of the cemetery (VARGA, MS. of Univ. doct. diss.). The bones are extremely light. On the

7. Grave 7 (Sen., female) — arthrosis deformans of the left femur and osteoporosis in the caput.
8. Grave 7 (Sen., female) — porosity of the left acetabulum.



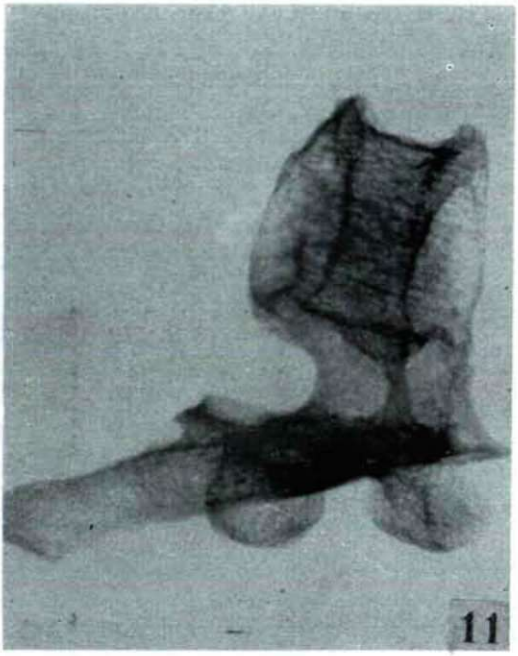

9. Grave 7 (Sen., female) — rarefied zones in the X-ray photograph of the unilateral pelvis.

atlas, the two arcus posteriores do not close, in the place of the tuberculum posterius a narrow gap is to be seen (Fig. 10); lumbar vertebra 5 is ossified with the sacrum.

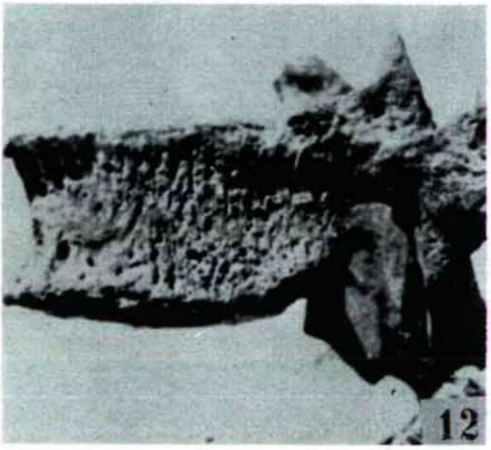
— spondylosis deformans;
— spondylarthrosis;
— coxarthrosis l. s ;
— osteoporosis;
— congenital anomalies: spina bifida atlantis posterior and sacralisatio.

Grave 9(Mat.-Sen., male): in the (dorsal) vertebrae the corpus depressed uniformly, ventral rarefaction (Fig. 11); fossa- and rim-formation (Fig. 12); foramina sacralia anteriora 2 are large and hollow, as compared with the others; in the proximal part of both radii and ulnae (Fig. 13), the tuberculum maius and minus of the right humerus, as well as the trochlea and capitulum humeri are strongly deformed; the fossa olecrani is extremely broad; the left caput humeri is flat, deformed; on both

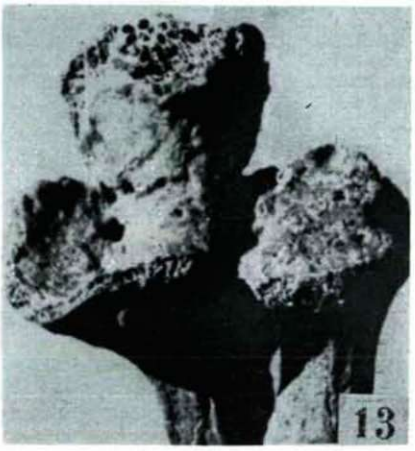
10. Grave 7 (Sen., female) — spina bifida atlantis posterior.



11. Grave 9 (Mat.—Sen., male) — rarefaction ib the X-ray photograph of the vertebra.



12. Grave 9 (Mat.—Sen., male) — spondylosis deformans.



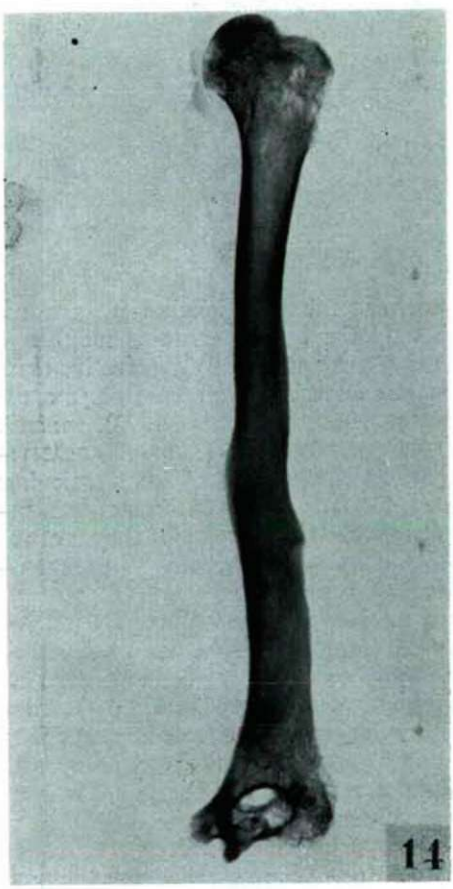
13. Grave 9 (Mat—Sen., male) — arthrosis deformans in the proximal part of the radius.

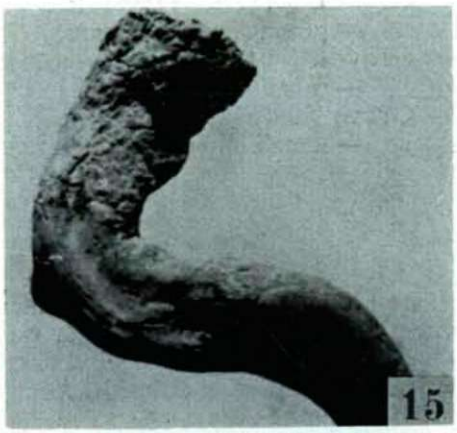
humeri, the zone above the epicondylus lateralis is broad and thin; the middle part of the right humerus is bent, on it there is an outlet (Fig. 14); both radii and fibulae are bent; the acromial extremity of the left clavicle is very broad and thin; on the

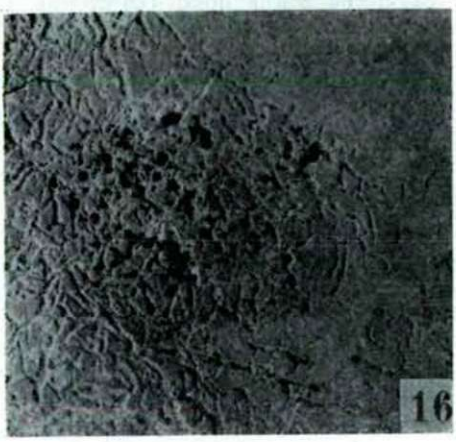
glenoidal cavity of the left scapula and its ventral rim spliting is to be seen; on the right clavicle, in the area between the corpus and the acromial extremity there is an "S"-shaped curvature (Fig. 15); by means of that, the length of clavicle is 3 cm smaller than that on the left.

— spondylosis deformans;
— arthrosis deformans;
— status post fracturam (clavicle);
— osteoporosis (osteoporomalacia?).

Grave 6 (Ad., male): osteophyte-formation at the rims of vertebrae.
— spondylosis deformans;







14. Grave 9 (Mat.—Sen., male) — status post fracturam in the left humerus.
15. Grave 9 (Mat.—Sen., male) — status post fracturam in the right clavicula.
16. Grave 8 (Mat.—Sen., female) — atrophia symm. senilis.
17. Grave 50 (Mat., female) — canalis sacralis apertus.

Grave 8 (Mat.-Sen., female): exostosis on the rim of vertebrae; symmetrical atrophic impression and porous zone in the parietal region of the cranium (Fig. 16).

— spondylosis deformans;
— osteoporosis;

Grave 9 (Sen., female): osteophyte-formation on the rim of vertebrae; rarefaction in the long tubular bones; the Ca/P quotient of bones is below the average value of the individuals of cemetera (VARGA, MS., univ. doct. diss.).

— spondylosis deformans;
— osteoporomalacia

Grave 20 (Mat.-Sen., male):
— spondylosis deformans;

Grave 30 (Sen., female):
— spondylosis deformans;

Grave 31 (Ad.-Mat., female):
— canalis sacralis apertus;

Grave 50 (Mat., female):
— sacralisatio et canalis sacralis apertus;

## Evaluation of the pathological deformations

Because of the diagnostic difficulties known in the palaeopathology, the particular diseases cannot be separated, we see only the bone symptoms manifesting only after a progress of the clinical picture being a result of the degenerative deformation of the articular disk. The formation of exostosis in vertebrae plays a part by the name of osteophytosis (MERBS—WILSON, 1960; CHAPMAN, 1972), generally however as spondylosis deformans (VYHNÁNEK, 1971; ACSÁDI—HARSÁNYI—NEMESKÉRI, 1962; TULSI, 1971); the degenerative deformations of facies articulares by the name of osteoarthritis (MARBS—WILSON, 1960) or spondylarthrosis (ACSÁDI—HARSÁNYI—NEMESKÉRI, 1962); while the characteristic change in shape of the articular ends of the long tubular bones as spondylosis deformans (NEMESKÉRI—HARSÁNYI, 1960; REGÖLY—MÉREI, 1964; 1965).

Spondylosis deformans can be observed in case of eight individuals. At four individuals of these — being of higher age of life (Mat.-Sen., resp. Sen.) but without the traces of any other articular disease — the phenomenon may be considered as a process connected with the age (JULKUNEN—LEHTOVIRTA, 1968). In these cases, on the basis of Chapman's paper (1972), the osteophyte-formation is of strenght III, resp. IV. Those in graves 6 and 49 are of younger age (Ad.), the exostosis-formation can, therefore, be regarded as a pathological deformation. (That in grave 6 is corresponding to strength III, that in grave 49 to strength II).

We have necessarily to emphasize the spondylitic deformation of the skeleton in grave 7 in spite of the old age of the individual (Sen.) as in the skeleton the traces of a multiple arthropathy can be seen: in the cervical vertebrae spondylarthritis, and in the articulatio coxae arthrosis deformans. Apart from the serious arthropathy, we have, however, to face the phenomenon of osteoporomalacia, as well. From the X-ray picture of the unilateral pelvis even myeloma multiplex could be

concluded but the cranium has no alteration at all and at the same time the diagnosis of osteoporomalacia is confirmed by the rerafaction seen in the long tubular bones, the porosity of acetabulum and caput femoris visible even to the naked eye, the lower value of the quotient Ca/P. In the paper of MERBS—WILSON (1960), we can see an osteoporotic vertebral surface corresponding to our case. According to HOLLÓ (1967), the arthrotic process can be connected with osteoporosis. In our case, of course, we cannot decide on the sequence problem of the two processes.

In skeleton No. 9, in additon to the typical deformity of the arthrosis deformans seen in the articulatio cubiti and articulatio radio-ulnar, the traces of fractures (right humerus and right clavicula) are remarkable.

The deformation noticed morphologically was confirmed by the X-ray picture: "A strong deformation of the right clavicula is visible as a result of a double fracture of the clavicula. The broken ends have a good junction, the osseous reconstruction is statisfactory. In the middle third part of the right humerus, a mild deformity of the bone may be observed as a result of a spiral fracture. In the same section of the bony substance a spotty aggregation of bone structure and spotty rarefaction appear, as well as a fistula, that may be evaluated as a trace of an osteomyelitis, supposedly being the result of fracture. "On the basis of the curvature of fibulae and radii (the pelvis missing unfortunately), as well as the deformations of vertebrae, and after studying Holló's paper (1967), we think on the process of osteoporomalacia. The fracture is a frequent concomitant of both processes (RATKÓCZY, 1959).

The symmetrical atrophy of the cranium of individual No. 8, as well as the rarefaction of the long bones of individual No. 10 can be regarded as an old-age osteoporosis. We find the symmetrical atrophy of cranium also in the paper of ACSÁDI—HARSÁNYI—NEMESKÉRI (1962), already mentioned.

A great may vertebrae of female No. 49 are missing. A block-formation as developmental anomaly may anyway be excluded: the vertebrae are not fully ossified, the osteal fossa is visible between them (REGÖLY—MÉREI, 1962), and there are to be taken into consideration the spondylitic origin (VYHNÁNEK, 1972), and the effect of micro-traumata and fractures, as well.

From among the developmental malformations, the bipartition of the foramen transversarium is represented in one, the spina bifida atlantis posterior in one, sacralisatio in two, canalis sacralis apertus similarly in two cases. In individual No. 50 we can see the joint occurrence of sacralisatio and canalis sacralis apertus, and in individual No. 7 that of sacralisatio and spina bifida atlantis posterior.

From among these developmental malformations, spina bifida atlantis posterior is the rarest one, more exactly it is studied the least (VYHNÁNEK, 1971). The spina bifida of epistropheus is mentioned by ÉRY (1968); the arcuate fissure of a lumbar vertebra is reported on by TULSI (1972), REGÖLY—MÉREI (1962). The most frequent developmental malformations observed in a palaeoanthropological material, are sacralisatio and canalis sacralis apertus (VYHNÁNEK, 1971; KENNETH A, BENNETH, 1972). FEREMBACH (1963) was describing the latter phenomenon under the name of spina bifida occulta.

In our material, from among the above characterized deformations the highest case-number is represented by spondylosis deformans and also the high number of osteoporotic cases and that of developmental malnutritions is remarkable. The malformations found, disintegrated to cases, are shown in Table 1.

*Table 1*

| Character of malformation | male | female | together |
|---|---|---|---|
| spondylosis deformans | 4 | 4 | 8 |
| arthrosis deformans | 1 | 1 | 2 |
| spondylarthrosis | — | 1 | 1 |
| osteoporosis (ostooporomalacia, sen. atrophia) | 1 | 3 | 4 |
| block vertebra | — | 1 | 1 |
| tatus post frakturam | 1 | — | 1 |
| congenital anomalies | 1 | 3 | 4 |
| total | 8 | 13 | 21 |

It is visible from this Table that the seven kinds of deformations are occurring numerically in 21 cases and affecting tne individuals (three males and seven females). VARGA (MS., univ. doct. diss.) carried out blood typing of the skeletons in the cemetery part, as well, according to which blood group B is prevailing in the series. (The distribution of the single blood types is given in Table 2)

*Table 2*

| A | B | 0 | AB | Total |
|---|---|---|---|---|
| 10 | 19 | 11 | 5 | 45 |
| (22,2 p. c.) | (42,6 p. c.) | (24,8 p. c.) | (11,1 p. c.) | |

On the basis of the cemetery map and keeping in view Fehér—Farkas's Table (1956) according to which, with full knowledge of the blood types, the person of father can be rendered probable or excluded, the establishment of relationship connections was attempted. In that way, three families were supposed taking place in the SW, N, and SE and NE parts of the cemetery. The family, more exactly the circle of relatives marked with No. 1, containing among others graves 6, 7, 8, 9, and 10, as well, are to be emphasized. These are located nearly semicircularly in the cemetery map and their relatedness seems to be confirmed by that in four of their individuals there are osteoporotic phenomena and rheumatoid spondylosis, and in case of the individuals of grave Nos. 7. and 9 there are the traces of serious arthropathy. On the basis of the blood type system, the individuals of graves 7 and 9 may be regarded as parents, the sister of one of them being the individual of grave 8 or 10, while grave 6 belongs to the second generation. Even if the expression "family" is too bold for that group, the identical pathological deformations anyway allow us to suppose the identical way of life. The osteophyte-formation of vetebrae is explained in a palaeoanthropological material mainly with the way of life of equestrian people (REGŐLY—MÉREI, 1962) but concerning the aetiology of the serious arthritic diseases also the humid environment may come into question (WATERMANN, 1971). And the value of the quotient Ca/P and the osteoporotic phenomena draw the attention to the non-satisfactory feeding, as well (HOLLÓ, 1967).

The semicircular continuation of the cemetery map described above is formed by graves 20, 30, and 31. That is to say, eight of the ten skeletons that are showing

the pathological deformations characterized by us are located semicircularly, following one another. In the skeletons of the other two relationship circles we have found no deformations although graves 49 and 50 are side by side, in the SE part of the cemetery.

All the graves described by us are containing grave furniture. The individuals of graves 7 and 9, who are showing the most serious deformations, are poor in grave furniture as compared with the average of the cemetery. Graves 6 and 20 showing only the deformation of spondylosis deformans are comparatively rich while the other six graves are mediocre, resp. poor ones in furniture.

## References


ACSÁDI, GY.—HARSÁNYI, L.—NEMESKÉRI, J. (1962): The population of Zalavár in the Middle Ages. — Acta Arch. Acad. Scient. Hung. *14*, 113—141.

CHAPMAN, F. H. (1972): Vertebral Osteophytosis in Praehistoric Populations of Central and Southern Mexico. — Am. J. Phys. Anthrop. *36*, 31—38.

ÉRY, K. (1968): Reconstruction of the tenth century Population of Sárbogárd on the Basis of Archaeological and Anthropological data. — Alba Regia. *8—9*, 93—147.

FEHÉR, M.—FARKAS, J. (1956): Szakértői bizonyítás a származásmegállapítási és a gyermektartási perekben (Evidence with experts in actions for establishing descent and affiliation). — Econ. and Legal Publishing House. Budapest.

FEREMBACH, D. (1963): Frequency of Spina occulta in Praehistoric Human Skeletons. — Nature. *199*, 100—101.

HOLLÓ, L. (1967): Osteoporosisok, kórélettan-klinikum (Osteoporoses, pathophysiological medicine). Budapest.

JULKUNEN, H. K. POYROALA—LEHTOVIRTA, F. (1968): Hyperostosis of the spine in relation to age and hyperglycaemia in men aged 30—39. — Annales Medicinae Internae Fenniae *57*, 1—7.

KENNETH, A. BENNETT (1972): Lumbo-sacral Malformations and Spina Bifida Occulta in a Group of Protohistoric Modoc Indians. — Am. J. Phys. Anthrop. *36*, 435—440.

LIPTÁK, P.—VARGA, I. (1974): Charakterisierung des anthropologischen Materials des awarischen Gräberfeldes von Kunszállás. — MFMÉ. 1971—2. 71—79.

MERBS, C. F.—WILSON, W. H. (1960): Anomalies and Pathologies of the Sadlermiut Eskimo Vertabral Column. — From National Museum of Canada, Bulletin No. 180. Contributions to Anthrop. *1*, 154—180.

NEMESKÉRI, J.—HARSÁNYI, L. (1959): Die Bedeutung palaopathologischer Untersuchungen für die historische Anthropologie. — Homo *10*, 203—226.

RATKÓCZY, N. (1959): Radiológia. (Radiology). Budapest.

REGÖLY—MÉREI, GY. (1962): Pelăopathológia II. Az ősemberi és későbbi emberi maradványok rendszeres kórbonctana (Palaeopathology II. Systematic pathological anatomy of the palaeoanthropic and later human remains). Budapest.

REGÖLY—MÉREI, GY. (1964): Paläopathologische Untersuchungen an Knochenfunden aus der Awarenzeit. — Sonderdruck aus Beiheft 1964 zur Schriftenreihe Geschichte der Naturwissenschaften, Technik und Medizin. Zum 60. Geburtstag von G. Harig. 270—279.

REGÖLY—MÉREI, GY. (1965): Alterserscheinungen an prähistorischen und mittelalterlichen Skeletten. — International Conference on Gerontology. Budapest. 129—135.

H. TÓTH, E. (1968): Archäologische Forschungen im Jahre 1967. (Völkerwanderungszeit) Kunszállás—Fülöpjakab. — Arch. Ért. *95*, 134.

H. TÓTH, E. (1971): Archäologische Forschungen im Jahre 1970. (Völkerwanderungszeit) Kunszállás—Fülöpjakab. — Arch. Ért. *98*, 275.

TULSI, R. S. (1972): Vertebral column of the Australian aborigine: selected morphological and matrical features. — Z. Morph. Antrop. *64*, 117—144.

VARGA, I. (1974): A kunszállási avarkori temető csontvázanyagának részletes metrikus, morfológiai és kémiai elemzése (A detailed metrical, morphological, and chemical analysis of the skeletal material of the Avar age cemetery at Kunszállás). — Manuscript of the University Doctora Dissertation, Szeged.

VYHNÁNEK, L. (1971): Analyse der Pathologischen Knochenbefunde aus der slawischen Begräbnisstätte von Bilina. — Anthrop. *9*, 129—135.
VYHNÁNEK, L. (1972): Die Blockwirbel in archäologisch geborgenem Skelettmaterial. — Anthrop: Anz. *33*, 258—266.
WATERMANN, R. (1971): Beobachtungen an Skeletten aus Gelduba. — Zeitschrift für Orthopädie und ihre Grenzgebiete *109*, 911—921.

Address of the authors:
Dr. J. VARGA
Dr. ANTÓNIA MARCSIK
Department of Anthropology
A. J. Univesity, H—6701 Szeged,
P. O. Box 428, Hungary

## Index

75-2847 — Szegedi Nyomda